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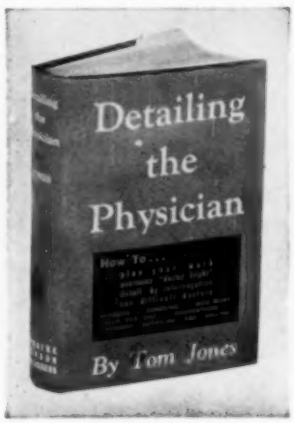

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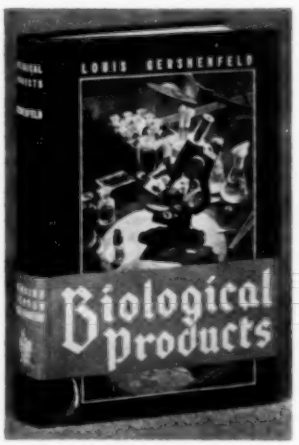

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Florida Volatile Oils. II.—*Mentha Piperita* L.*

By P. A. Foote† and A. W. Matthews‡

In 1930, Dr. B. V. Christensen, Director of the School of Pharmacy, University of Florida, reported to the 4th Annual Symposium of the Genus *Mentha* (1) that a supposedly true type of *Mentha piperita* L. grown in the University's Medicinal Plant Garden the previous season had yielded an oil which assayed only 7.23% menthol and contained about 80% pulegone. He pointed out that the plant may have undergone dehybridization or a delayed reduction in the process of metabolism and expressed the hope that further study might explain the low menthol content of peppermints grown in the South.

This report was apparently based on the work of Hiner (2) who investigated oils produced in 1929 and 1930 from Florida-grown peppermint. Only the physical and chemical constants and the ketone content, as indicated by bisulfite assay, were determined at that time.

Since several of the peppermint oils of the years 1929–1932, inclusive, were still available, it was felt that a more detailed study of their composition and especially of the non-pulegone constituents should be undertaken. It is hoped that data provided by determining and reporting the other changes which accompanied the failure to develop menthol in these abnormal oils may prove of some value to others in their study of the biogenesis of peppermint oil and the genetics of the genus *Mentha*.

The question of the possibility of mistaken identity immediately suggests itself when the abnormal character of these oils is considered. However, the original root stock was obtained from the Bureau of Plant Industry, Washington, D. C., and in 1930 specimens of the plants then growing in the medicinal plant garden at the University of Florida were sent to Washington for verification. Reports from Messrs. Sievers and Lowman stated that no mistake was probable since the specimens from Florida

* Abstract of a portion of a thesis submitted to the Graduate Council of the University of Florida in partial fulfilment of the requirements for the degree of Doctor of Philosophy, July 1941.

Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

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Table I.—Peppermint Oils 1929–1932

Sample No.	Origin of Root Stock	Date Distilled	Green Herb (Lbs.)	Yield, Cc.	Yield, Lbs. per Acre
2	Washington, D. C.	6/25/29	525	1330	36.03
3	Washington, D. C.	7/ 7/30	1065	1620	25.40
4	Washington, D. C.	10/28/30	400	675	11.86
5	Washington, D. C.	8/ 7/31	55	115	...
6	Oregon	8/12/32	316	630	...
7	Washington, D. C.	9/26/29	682	1230	33.15

Sample No. 1 of this series, a Japanese Peppermint Oil, was not included in this investigation.

Table II.—Physical and Chemical Constants

Sample No.	$d_{25°}^{25°}$	$n_D^{20°}$	$[\alpha]_D^{25°}$	A. V.	E. V. (Calc. as Menthyl Acetate)	E. V. after Acetyl. (Calc. as Free Menthol)	Ketone Content, Per Cent
2	0.9275	1.4838	+13.65°	2.33	13.10 (4.63%)	36.49 (8.55%)	87
3	0.9251	1.4865	+15.81°	2.31	13.20 (4.67%)	19.70 (4.89%)	88
4	0.9311	1.4860	+16.96°	2.10	10.91 (3.87%)	43.95 (9.49%)	88
5	0.9298	1.4871	+15.63°	1.15	10.35 (3.66%)	30.23 (5.68%)	86
6	0.9192	1.4670	+ 0.24°	2.04	30.94 (10.94%)	138.00 (33.44%)	3
7	0.9351	1.4880	+17.41°	2.28	11.68 (4.13%)	35.58 (6.82%)	89

compared exactly with original stock growing in the Bureau's garden and producing oils of relatively high menthol content.

In this study a contemporary oil produced from peppermint grown from Oregon root stock is also included. The particulars with respect to the origin of the several samples are given in Table I.

EXPERIMENTAL

Physical and Chemical Constants.—For each of the samples these were redetermined and the values obtained set out in Table II. The E. V. after acetylation were determined according to the U. S. P. XI. In the latter, however, the calculation was carried out as recommended by Gildemeister and Hoffmann (3), *i. e.*, % free menthol = menthol in original oil $\times (1 - (E_1/E_2))$. The ketone content was determined by the neutral sulfite method.

Apparent Ester Value, Pulegone.—In 1929, Werner (4), working with the oil of *Pycnanthemum muticum* (*Koellia mutica* Michx.), an oil found to be rich in pulegone, observed that the presence of this constituent led to abnormally high ester and free alcohol values. Upon investigation he found that pulegone itself gave apparent ester values both before and after acetylation. To check this observation a sample of pulegone, regenerated from a sulfite addition product obtained with oil No. 2, was assayed by the U. S. P. XI method with the following results: E. V., 7.02, 7.39 (av. 7.21); E. V. after acetylation, 34.80, 33.14, 34.14, 33.19 (av. 33.82).

Consequently it would appear that menthyl acetate and free menthol contents as determined by the U. S. P. XI method (see Table II) cannot be relied upon in the case of oils high in pulegone content.

Brignall Menthol Assay.—Brignall (5) has recently devised a rapid method for determining free menthol which is claimed to avoid some of the errors of the official method. Its application to these pulegone-containing oils was studied and the values so obtained are compared with those from the official method in Table III.

Table III.—Comparison of Brignall Method and U. S. P. XI Method

Sample No.	Percentage Free Menthol: Average by Brignall Method	Average by U. S. P. Method
2	9.01	8.55
3	2.46	4.89
4	5.60	9.49
5	6.83	5.68
6	26.08	33.44
7	2.58	6.82

Although the results of the Brignall determinations are generally lower than those obtained by the U. S. P. method, this may be due to the elimination of errors other than the error due to pulegone. This is illustrated particularly by the figures shown for oil No. 6, which contained only 3% pulegone. In an effort to determine the extent of the error due to pulegone, the study of the Brignall method was extended to known mixtures:

1. To 1.0315 Gm. of oil No. 6, 0.7039 Gm. of regenerated pulegone was added and the determination of free menthol carried out. Found: 26.54% (av., Table III, 26.08%).

2. A mixture was prepared containing menthol 30 parts, pulegone 30 parts, menthone 20 parts, and *d*-limonene 20 parts (all by weight). This was then assayed by the Brignall method and the results compared with determinations using a 30% w/w solution of menthol in *d*-limonene as a control. The following free menthol values were found:

For menthol (30%) in *d*-limonene..	30.74, 29.06
For menthol (30%)-pulegone-menthone mixture.....	27.28, 29.80

Thus in no instance in which pulegone was added did the Brignall method yield a result which was significantly higher than the control.

Table IV.—Re-fractionation of Oil No. 2

Fraction[a]	Vol., Cc.	B. P., °C., 5 Mm.	B. P., ° C., 760 Mm.	$d_{25°}^{25°}$	$n_D^{20°}$	$[\alpha]_1^{25°}$	Ketone, Per Cent[b]
Fraction 2							
I	4	48–50	155.4	0.8542	1.4659	−16.0°	
II	5	55–58	169	0.8413	1.4712	−48.8°	
Fraction 3							
I	1	60	170.4	....	1.4774	...	
II	5	76–80	214.2	0.9227	1.4792	+18.8°	
III	2	84	211.4	0.9636	1.4849	...	
Fraction 4 (*a*)							
I	12	78–79	221	0.9291	1.4816	+23.2°	88
II	40	81	221	0.9287	1.4832	+22.3°	46
III	164	83	221	0.9311	1.4850	+21.0°	94
Fraction 4 (*b*)							
I	67	80	221	0.9384	1.4849	+21.0°	97
II	25	81.5	222	0.9388	1.4860	+16.0°	93
Residue (Original)							
I	11	82		0.9347	1.4843	+18.2°	94
II	32	87–88		0.9372	1.4860	+14.0°	88
III	3.5	94		...	1.4888	+ 6.6°	..
IV	3.0	101		...	1.4962	− 8.0°	59
Residue							

[a] Fraction 1 (4 cc.) was received in the cold trap during the original fractionation and was not re-fractionated.
[b] Neutral sulfite method.

Table V.—Re-fractionation of Oil No. 3

Fraction	Vol., Cc.	B. P., ° C., 5 Mm.	B. P., ° C., 760 Mm.	$d_{25°}^{25°}$	$n_D^{20°}$	$[\alpha]_D^{25°}$	Ketone, Per Cent
Fraction 1 (Received in cold trap, not re-fractionated)							
	3.4	Below 30		0.8581	1.4680	−26.8°	
I	2.5	38	157	0.8556	1.4490	−16.8°	
II	7.5	44	168–170	0.8402	1.4706	−55.2°	
Fraction 3							
I	310	83– 85	220–223	0.9332	1.4859	+23.2°	98.5
Fraction 4							
I	13	91		0.9380	1.4892	+12.4°	86
II	5	91–114		0.9451	1.4989	− 4.0°	38.3
III	3.6	114–138		0.9649	1.5025	− 2.0°	
Residue							

Fractional Distillation of Washington, D. C., Oils.—Five hundred cc. of oil No. 2 was shaken with 5% Na_2CO_3 to remove free acids, washed with water and dried over anhydrous Na_2SO_4. It was then fractionated at 20 mm. using an 18-inch column packed with small Berl saddles and refractionated at 5 mm. In the re-fractionation either a 6-inch water-jacketed Vigreaux 24–40 standard taper column or an 18-inch 24–40 standard taper column packed with Penn State rings was used, depending on the size of the original fraction. The results of re-fractionation are given in Table IV.

After removing free acids, 380 cc. of oil No. 3 (Table I) was fractionated at 5 mm. using the 18-inch column packed with Penn State rings and then re-fractionated at the same pressure, as indicated above. The data with respect to the fractions obtained are set out in Table V.

Referring to Table I, the remainder of oil No. 3 (200 cc.), oil No. 4 (500 cc.) and oil No. 7 (500 cc.) were each treated for several days with a large volume of 30% solution of sodium bisulfite until the ketone addition reaction appeared to be complete. The unabsorbed portions, after washing and drying,

Table VI.—Fractionation of Mixture of Unabsorbed Oils

Fraction	Vol., Cc.	B. P., ° C., 20 Mm.	B. P., ° C., 760 Mm.	$d_{25°}^{25°}$	$n_D^{20°}$	$[\alpha]_D^{25°}$	Free Alcohol, Per Cent[a]
1	4	58	168.5	0.8467	1.4679	−47.04°	...
2	4	63	171	0.8399	1.4675	−68.84°	...
3	6	81	201	0.8893	1.4559	+10.40°	14.04
4	4	88–89	208–210	0.9080	1.4622	+ 4.32°	44.10
5	4	...	209–211	0.9267	1.4750	+ 2.12°	39.93
Residue							

[a] Brignall Method (5) (calc. as menthol).

Table VII.—Fractionation of Oil No. 6

Fraction	Vol., Cc.	Per Cent of Whole	B. P., ° C., 20 Mm.	B. P., ° C., 760 Mm.	$d_{25°}^{25°}$	$n_D^{20°}$	$[\alpha]_D^{25°}$
1	6.3	2.74	Below 30[a]	165	0.8647	1.4681	+23.68°
2	4.0	1.74	58–59	171	0.8855	1.4650	−13.00°
3	9.5	4.13	62–63	175	0.8804	1.4641	−26.64°
4	16.2	7.04	82–83	206.5	0.9542	1.4787	+75.89°
5	24.0	10.43	83–85	208–209	0.9373	1.4711	+47.50°
6	33.0	14.35	86–87	211–212	0.9046	1.4570	+ 9.88°
7	5.7	2.42	91	213–214	0.9029	1.4618	+16.16°
8	66.8	29.04	95–97	218	0.9091	1.4689	−16.16°
9	25.0	10.87	97–101	228.5	0.9184	1.4681	−16.96°
Residue	25.0	10.87	...	...	...	...	...
Total	216.8						

[a] Received in the cold trap.

Table VIII.—Comparison of Physical Constants of Separated Ketone with Those Recorded for Pulegon

	B. P., ° C.	d		n_D		$[\alpha]_D$
Averages found	220–223	$d_{25°}^{25°}$	0.9329	$n_D^{20°}$	1.4850	+21.60°
Grignard and Savard (7)	84/6 mm.	$d_{4°}^{15°}$	0.9346	n_D	1.4894	$[\alpha]_D$ +21.00°
Heilbron (8)	224	$d_{4°}^{15°}$	0.9370	$n_D^{15°}$	1.4880	$[\alpha]_D$ +28.23°
Kon (9)	109/19 mm.	$d_{4°}^{16.9°}$	0.9381	$n_D^{16.9°}$	1.4881	
Schimmel and Co. (3)	224	$d_{15°}$	0.9405	n_D	1.48796	+20.48°
Wallach (3)	221–223	d	0.9360	n	1.4846	

Table IX.—Fractionation of Pulegone (250 Cc.) from Oil No. 5

Fraction	Vol., Cc.	B. P., ° C., 20 Mm.	B. P., ° C., 760 Mm.	$d_{25°}^{25°}$	$n_D^{20°}$	$[\alpha]_D^{25°}$
1	2	95	209.5	0.9162	1.4672	+59.64°
2	2	99	216	0.9211	1.4697	...
3	4	99–101	220	0.9239	1.4727	+38.56°
4	4	101	222	0.9245	1.4786	+30.20°
5	8	102	217	0.9294	1.4836	+26.04°
6	4	102–103	222.5	0.9316	1.4849	+26.16°
7	6	104	222.5	0.9291	1.4811	+34.68°
8	18	105–106	223	0.9315	1.4853	+26.88°
9	87	105–106	224.5	0.9321	1.4681	+24.99°
10	75	106–107	222	0.9315	1.4870	+22.54°
Total	228					

were mixed together and 82 cc. of this pulegone-free mixture was fractionated at 20 mm. (Table VI). An efficient 4½-foot spiral screen packed column, described by Lecky and Ewell (6) was employed.

Fractional Distillation of Oil No. 6 (Oregon).—Two hundred and thirty cubic centimeters of this oil, the physical and chemical constants of which differed markedly from the Washington, D. C., oils (see Table II), was taken for fractionation at 20 mm. The 4½-foot spiral screen packed column was used and the fractions listed in Table VII were obtained.

Fractional Distillation of Pulegone.—In Table VIII the average physical characters of the several lots of ketone separated from the Washington, D. C., oils are compared with those recorded for pulegone by different authors. The ketone was obtained either by fractionation or by regeneration from bisulfite addition product, or both.

Each of the pulegone lots was fractionally distilled using the 4½-foot screen packed column mentioned above. The data presented in Table IX may be considered typical of such fractionations.

IDENTIFICATION OF CONSTITUENTS

I. Terpenes and Other Low-Boiling Constituents.—As indicated in Tables IV, V and VI, only small portions of the fractions containing these constituents were available from the Washington, D. C., oils.

The constants of fraction 2, I (Oil No. 2) and fraction 2, I (Oil No. 3) checked reasonably well with those reported for α-pinene. (Gildemeister and Hoffmann (3) for l-α-pinene: b. p. 153.5° to 154.5° C.; $d_{16°}^{19°}$ 0.8626; $n_D^{13°}$ 1.4690; $[\alpha]_D$ −43.8°. Heilbron (8): b. p. 155° to 156° C.; $d_{20°}$ 0.8595; $n_D^{20°}$ 1.47299; $[\alpha]_D$ −47.2°.) A nitrosochloride (m. p. 103° to 105° C.) was prepared, which yielded a nitrol-piperidide (m. p. 121° to 122° C.). These derivatives were considered to have resulted from the presence of the inactive α-pinene.

The fractions boiling 168° to 171° C. were examined for limonene and phellandrene but neither the tetrabromide nor the nitrosite could be obtained.

Cineol: Fraction 1 from the mixture of oils Nos. 3, 4 and 7 (see Table VI) smelled strongly of cineol and suffered a loss of 26% when shaken with 50% resorcin solution.

1,3-Methylcyclohexanone: During the fractionation of one of the lots of pulegone a small amount of oil was recovered from the cold trap. Its characters were determined as follows: m. p. 168° to 169° C.; $d_{25°}^{25°}$ 0.9121; $n_D^{20°}$ 1.4480. It yielded a crystalline semicarbazone (m. p. 178° to 179° C.). Wallach (10) records for 1,3-methylcylcohexanone: b. p. 169° C.; $d_{21°}$ 0.909; n_D 1.4460; $[\alpha]_D$ +12.5°; semicarbazone 180° C.

Cineol was also found to be present in the low-boiling fractions from oil No. 6 (Oregon). Fractions 2 and 3 (see Table VII) combined to the extent of 57% and 41%, respectively, with resorcin solution. In this case a small amount (less than 1 cc.) of a colorless oil was recovered from the combined resorcin addition mixtures. It smelled strongly of cineol and the following characters were determined: b. p. 171° to 172° C.; $n_D^{20°}$ 1.4588. Simonsen (7) records for cineol: b. p. 174.4° C.; $n_D^{15°}$ 1.45839. The quantity available did not permit the preparation of a derivative.

II. Menthone and Free Menthol.—With respect to the Washington, D. C., oils, the only fractions at all suggestive of either menthone or free menthol were obtained from the fractionation of the residue uncombined with bisulfite from the mixture of oils Nos. 3, 4 and 7. Fractions 3, 4 and 5 (see Table VI) possessed a similar penetrating odor suggestive of menthol, but masked by a sweetish, though somewhat musty smell. By the Brignall method their respective free alcohol contents, calculated as menthol, were 14.04%, 44.1% and 39.93%.

Menthone: The constants recorded in Table VI for fraction 3 approach those given (3) for menthone: b. p. 208° C.; d 0.8940; n_D 1.4496. This fraction yielded a semicarbazone which melted 181 to 182° C. Melting points ranging from 184° to 186° C. are reported for menthone semicarbazone by various authors.

Menthol: Fractions 4 and 5 (Table VI) gave phenylurethanes which crystallized from pentane in spear-shaped masses of slender crystals (m. p. 111° to 112° C.). Menthyl phenylurethane is usually given as 111° to 112° C. The reaction mixtures also gave smaller yields of a phenylurethane which appeared to be more soluble in pentane and crystallized as short translucent rods (m. p. 107° to 108° C.). Gildemeister and Hoffmann (3) cite 107° to 108° C. as the melting point of *d*-neomenthol phenylurethane. Menthol was also identified in these fractions through its 3,5-dinitrobenzoate, m. p. 152° to 153° C. For the same Cohen and Armes (11) give 153° to 154° C.

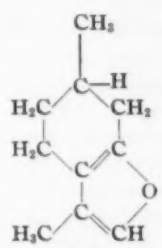
Oil No. 6 (Oregon), in contrast to the Washington oils, gave well-defined menthone and menthol fractions at the expected levels. From fractions 6 and 7 (Table VII) good yields of a semicarbazone (m. p. 187° C.) were obtained. When hydrolyzed by treatment with oxalic acid and steam distillation, a light yellow oil was obtained. It has a menthol-like odor and the following constants: b. p. 209° to 210° C.; $d_{25°}^{25°}$ 0.8900; $n_D^{20°}$ 1.4509; $[\alpha]_D^{25°}$ −13.76. Thus menthone was established as a major constituent of these fractions. Menthol was separated by freezing out from fraction 8 and identified through its phenylurethane and 3,5-dinitrobenzoate. In this case an α-naphthylurethane (m. p. 117° to 119° C.) was also prepared. Bickel and French (12) record 119° C.

III. Identification of 3,6-Dimethylcoumarone Tetrahydride (Menthofurane).—Fractions 4 and 5, oil No. 6 (Oregon), possessed boiling points almost within the menthone-menthol range and yet the observed rotations of +75.89° and +47.50°, respectively, pointed to the presence of some other constituent. These fractions apparently contained some menthone, however, and this was removed as the semicarbazone, leaving a thick, light brown oil.

Carles (13), in 1929, reported the isolation from peppermint oil of a new constituent possessing the following constants: b. p. 196° C./760 mm.; $d_{15°}$ 0.965; $n_D^{20°}$ 1.4807; $[\alpha]_D$ +81°. It was later identified by Wienhaus and Dewein (14) who derived its empirical formula, $C_{10}H_{14}O$, and established its constitution as 3,6-dimethylcoumarone tetrahydride (4:5:6:7). It was named "Menthofurane."

The specific gravity of 0.9542 found for the fraction having a plus rotation of +75.89° pointed to the possibility that 3,6-dimethylcoumarone tetrahydride was a constituent of this oil. It was also noted that colorless crystals tended to form above the surface of the liquid on the glass wall of the test-tube in which the fraction was stored. A small yield of similar crystals was obtained by oxidizing 2 cc. of the fraction with chromic acid. The melting point in each case was found to be 183° to 185° C. Wienhaus and Dewein state that the substance 3,6-dimethylcoumarone tetrahydride is oxidized in air to a colorless acid melting at 186° C.

IV. Proof of Pulegone.—The physical characters of the well-defined ketone fractions from the Washington, D. C., oils have been averaged in Table VIII and compared with those reported for pulegone. Pulegone was further characterized by preparation of derivatives and by hydrolysis.



Menthofurane

Pulegone semicarbazone: Good yields of semicarbazone were obtained when these ketone fractions were treated with semicarbazide. The impure semicarbazone melted at 158° to 166° C. Recrystallization from alcohol failed to raise this above 171° C. However, when the procedure of Hugh, Kon and Linstead (15) was applied, the semicarbazone was obtained in the form of translucent crystals melting at 173° to 174° C. (Recorded for pulegone semicarbazone: Heilbron (8) 174° C., Gildemeister and Hoffmann (3) 167.5° to 168° C.)

Pulegone 2,4-dinitrophenylhydrazone: The method of Brady (16) was used in the preparation of this derivative. The red granular substance so obtained

was recrystallized from alcohol and was then found to melt rather indefinitely at 142° C. After recrystallization from pentane it was obtained in the form of dark red crystalline plates, m. p. 147° to 148° C. Campbell (17) gives 147° C. while several others record 142° C.

Hydrolysis of pulegone: Eighty-two cubic centimeters of regenerated pulegone (from oil No. 2) was hydrolyzed by boiling with an equal volume of 85% formic acid in an oil bath for 16 hours. The oil separated from this reaction mixture was dried and fractionated. A 17-cc. fraction had the following constants: b. p. 163.5° C.; $d_{25°}^{25°}$ 0.9190; $n_D^{20°}$ 1.4456; $[\alpha]_D^{25°}$ +12.2°; m. p. (semicarbazone) 179° to 180° C. These results show good agreement with the values recorded by Wallach (see above) for 1,3-methylcyclohexanone.

Oil No. 6 (Oregon) was also found to contain a small amount of ketone other than menthone (see Table II). Pulegone was identified through its semicarbazone (m. p. 171° to 172° C.) as a minor constituent of fraction 9 from this oil.

V. Identification of d-Isomenthone.—During the preparation of pulegone semicarbazone, it was found that some other reaction product of relatively high melting point was always present in the first separation of crystalline material. After all, or the greater part, of the pulegone semicarbazone had been separated, excessive dilution of the reaction mixture always resulted in a small yield of white granular material which melted indefinitely between 125° and 150° C. The high-melting material appeared to be quite insoluble in alcohol and repeated washing with pentane and ether followed by digestion with boiling alcohol left it in the form of a residue (m. p. 256° to 260° C.). Thus the possibility of hydrazodicarbonamide (m. p. 245° to 246° C.) appeared to be excluded.

In 1907, Barrowcliffe (18), working with oil of American Pennyroyal (*Hedeoma pulegioides* (L.) Pers.), reported circumstances similar to those noted above. After regenerating the ketone from the lower-melting semicarbazone and determining its properties, he concluded *d*-isomenthone was a constituent of the oil under examination. While Barrowcliffe does not appear to have connected the low-melting semicarbazone with the material insoluble in hot alcohol, it is to be noted that Heilbron (8) records a melting point of 264° C. for *d*-isomenthone semicarbazone. It seems likely, therefore, that this ketone is capable of forming both α- and β-semicarbazones.

VI. Examination of High-Boiling Residues.—In the case of the Washington, D. C. oils, the examination of the still residues boiling above the pulegone range (220° to 223° C.) was restricted by the relatively small bulk of such residues and the fact that a considerable amount of resinous material was present. This latter probably arose as a result of prolonged heating of the pulegone during fractionation.

The high-boiling residue from oil No. 6 (Oregon), while also small in quantity, was relatively free from resin. Since the assay of the original oil had indicated the presence of 10.94% ester (calculated as menthyl acetate), this residue was saponified and the oil recovered from the saponification mixture was distilled over an oil bath at 760 mm. A small amount of an oil was obtained: b. p. 206° to 212° C.; $d_{25°}^{25°}$ 0.9020; $n_D^{20°}$ 1.4683; $[\alpha]_D$ −22.36°. When assayed by the Brignall method, 75.42% of free alcohol (calculated as menthol) was found.

Menthol was identified in this case as the phenylurethane (m. p. 112° C.) and the 3,5-dinitrobenzoate (m. p. 154° C.).

DISCUSSION OF RESULTS

The abnormal character of the Washington, D. C., oils is apparent from their physical and chemical constants, as set out in Table II. The characters for the five samples studied may be averaged as follows: $d_{25°}^{25°}$ 0.9297; $n_D^{20°}$ 1.4863; $[\alpha]_D$ +15.89°; ester content (as menthyl acetate) 4.19%; free alcohol content (as menthol) 7.09%; ketone content 88%. It has been demonstrated, however, that ester and free alcohol contents determined by the U. S. P. XI method cannot be relied upon in the presence of large amounts of pulegone. The apparent ester value due to pulegone is explainable on the basis that undue resinification or polymerization is induced by prolonged heating with potassium hydroxide, some of the base being used up. In the case of the free alcohol determination, the formation of an enol acetate during acetylation where pulegone is present would appear to explain the higher ester value. Difficulty in judging the end-point in saponification mixtures was also a factor when the U. S. P. method was used.

When the Brignall method was applied to these same oils, an average of 5.30% free alcohol (calculated as menthol) was found. Furthermore when the quantity of menthol present was known and pulegone was added, the Brignall method was found to give a satisfactory result. The fact that saponification after acetylation is avoided in this procedure is believed to have been a decided advantage in the case of these pulegone-containing oils.

The oil produced from peppermint grown from Oregon root stock stood intermediate in respect to all its characters between the Washington, D. C., oils and U. S. P. stand-

ards. A free alcohol content (calculated as menthol) of 26.08% was found by the Brignall method, as compared to 33.44% by the U. S. P. XI method. Since any error due to pulegone must necessarily be very small in this case, there is evidence to support Brignall's contention that other errors are avoided in this method.

The low menthol content and high ketone content of the Washington, D. C., oils, as indicated by assay, was substantiated by the results of fractionation. The data in Tables IV and V hold no suggestion of either menthone or menthol. However, menthone was found in traces in Fraction 3, Table VI. In this case a total of 1200 cc. of oils Nos. 3, 4 and 7 was represented in the fractionation. Fractions 3, 4 and 5 from this same mixture also contained free alcohol. Brignall assays indicated 14.04, 44.1 and 39.93%, respectively (calculated as menthol). Menthol was identified in these fractions but the amount was well below even the average of 5.30% found in the original oils by the Brignall method. There was some evidence that traces of *d*-neomenthol also accompanied the menthol.

With oil No. 6 (Oregon), on the other hand, well-defined menthone and menthol fractions were obtained at the expected levels. Furthermore the total quantity of menthol separated by fractionation (see fractions 8 and 9, Table VII) was an approximation of the amount indicated by assay. 3,6-Dimethylcoumarone tetrahydride (or menthofurane) was also found to be associated with menthone and menthol in this oil. Its high dextro-rotation and the close approach of its boiling point to that of menthone are believed to have been responsible for the dextro-rotatory power observed for the menthone fractions (Fractions 6 and 7, Table VII). The low rotation of the menthol fractions, only $-16°$ approximately, may likewise have been due to contamination by menthofurane, although the presence of small amounts of *d*-neomenthol would influence this also.

Pulegone was identified as the major constituent of the Washington, D. C., oils and as a minor constituent of oil No. 6, which was produced from peppermint grown on the same soil but from root stock of quite different origin (*i. e.*, Oregon). From the standpoint of biogenesis, the latter fact may hold equal significance with the former.

Fractional crystallization of the semicarbazones obtained from various pulegone fractions revealed that *d*-isomenthone was closely associated with pulegone in the Washington, D. C., oils. Furthermore, the relatively high degree of efficiency of the spiral screen packed column resulted in the separation from apparently pure pulegone of small amounts of oil with dextro-rotations ranging up to 59.64°. When these fractions were mixed and treated with semicarbazide, a white solid material (m. p. 135° to 142° C.) separated which resembled the low-melting semicarbazone obtained by fractional crystallization. Both of these reaction products resisted efforts toward recrystallization which is reported to be characteristic of *d*-isomenthone semicarbazone.

The examination of the low-boiling and high-boiling portions of the oils was generally less satisfactory because of the small amounts of such fractions available. Inactive α-pinene was identified and another terpene of higher boiling range was also present. Cineol was also found to be a constituent of both the Washington, D. C., and Oregon oils. Identification of menthol in the saponification residue indicated that menthyl esters were present in the latter oil.

SUMMARY

Several abnormal peppermint oils produced during the years 1929–1932, inclusive, have been studied. These oils were distilled from plants grown from root stock originally obtained from Washington, D. C. A contemporary oil obtained from plants grown from Oregon root stock has also been examined.

The Washington oils have been found to consist mainly of pulegone with menthol and menthone present only in traces. The Oregon oil, although well below the official standard, contained a good proportion of menthol. It also contained a small amount of pulegone.

In connection with the quantitative determination of free menthol, it has been

established that with the Brignall method there is less tendency toward the formation of enol acetates when pulegone is present than there is when the U. S. P. procedure is employed. The elimination of saponification after acetylation is also a factor in favor of the Brignall method when oils containing pulegone are being assayed.

The following constituents from among the twenty-two listed (3) for American peppermint oil have been identified in these oils: inactive α-pinene, cineol, *l*-menthone, *l*-menthol (both free and combined), pulegone (and its hydrolysis product 1,3-methylcyclohexanone).

The presence of an appreciable amount of 3,6-dimethylcoumarone tetrahydride has been detected in the Oregon oil. This constituent, first reported in 1930, is characterized by a high degree of dextro-rotatory power, a relatively high specific gravity and a rather unpleasant odor.

Small amounts of *d*-isomenthone have been found associated with pulegone in the Washington, D. C., oils. Pulegone and *d*-isomenthone are also reported to have been found together in the oil from the quite unrelated *Hedeoma pulegoides* (American Pennyroyal).

d-Neomenthol is thought to have been present in small amounts. The odor of the saponification residues in particular suggested this isomeric menthol, but identification was not conclusive.

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Florida Volatile Oils.* III.—*Pycnanthemum Muticum* (Michx.) Pers. Assay Methods and Minor Constituents

By P. A. Foote† and Domingo Donate Torres‡

The volatile oil of *Pycnanthemum muticum* (Michx.) Pers. also known as *Koellia mutica* (Michx.) Britton or Mountain Mint was reported on by Werner (1) in 1931. He identified pulegone 62–72%, *d*-menthol 15%, *d*-menthone 8%. He also found an unidentified ketone, traces of acetic and formic acids, a small amount of a non-volatile acid and an indication of terpenes and sesquiterpenes. Since then plots have been continually cultivated in the Florida Medicinal Plant Garden and recently in different sections of the state. The oil has aroused much

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interest because of its high pulegone content which may be utilized for the production of menthol by hydrogenation. It can also be used as a satisfactory substitute for oil of Pennyroyal. Therefore it seemed worth while (*a*) to improve the method of assay for pulegone which has been long and tedious and has not given uniform results in the hands of many workers; (*b*) to determine the physical and chemical properties of this oil from different plantings; (*c*) to redetermine its non-pulegone constituents.

Gildemeister (2) states that although attempts have been made repeatedly to work out a general method for the determination of aldehydes and ketones, this problem has not yet been solved in a satisfactory manner. Failure has been due to the fact that not all aldehydes and ketones react equally well with a given reagent, but that in each case only a few yield quantitative results.

The bisulfite method for the assay of ketones, introduced first in 1890 by Schimmel and Co. (3), was selected for this investigation. On this particular oil it requires as much as 15 to 20 hours. Several modifications of this method were made in an effort to make it more rapid and efficient. As reported by Werner (1) menthone occurs in this oil, but it is well known that this ketone does not combine with acid sulfite.

Gildemeister (2) advises that too much free sulfurous acid retards the reaction and recommends neutralizing it with sodium carbonate. This we have found necessary. Baeyer (4) recommends the addition of 25% of ethyl alcohol to an oil to assist the combination of a ketone with the acid sulfite. This did not prove satisfactory. Neither did such solvents as dioxane, propylene glycol, diethylene glycol, ethylene glycol, propylene glycol laurate and pyridine. Different percentages of ethyl alcohol were then tried. Best results were obtained by using 10 cc. of ethyl alcohol with 10 cc. of oil and neutralizing the excess sulfur dioxide with sodium hydroxide. When the bisulfite method was so modified it was found to be superior to the hydroxylamine method and lowered the time of pulegone assay to five hours.

Florida climatic conditions permit two crops annually. Three different lots of oil were used, each from the first seasonal crop. Two were produced in the Florida Medicinal Plant Garden in 1939 and 1940. One was from the 1940 crop produced by the United States Sugar Corporation at Clewiston, Florida. Second crops were not distilled but used for propagation.

The physical and chemical properties and pulegone content of the three lots of oil did not differ materially. In addition to the constituents identified by Werner (1) limonene was found.

EXPERIMENTAL

I. ASSAY METHODS

1. General Bisulfite Method.—See Gildemeister (2). A 10-cc. sample of the 1939 crop when treated by this method and heated for eight hours gave only 8% (pulegone) absorption by volume.

2. Neutralization of Free Sulfurous Acid.—The residual oil was again treated as above except that the free sulfurous acid was neutralized with NaOH T.S. after the oil and the bisulfite solution were mixed in the flask. Phenolphthalein was used as an indicator. While heating the solution turned red due to NaOH set free which was neutralized from time to time with 30% $NaHSO_3$ solution. After six hours of heating 65.9% additional (pulegone) absorption took place.

3. Effect of Ethyl Alcohol.—To 10 cc. of the 1939 oil 2 cc. of ethyl alcohol was added and the general bisulfite method repeated without neutralizing the free sulfurous acid. After eight hours of heating 16% (pulegone) was absorbed.

4. Effect of Alcohol and Neutralization.—(*a*) The residual oil from the last procedure was again extracted with 30% bisulfite solution neutralizing from time to time the free sulfurous acid with NaOH T.S. After six hours of heating 53% more ketone (pulegone) was absorbed.

(*b*) Four 10-cc. samples of the 1939 oil were each diluted with 10 cc. of ethyl alcohol and treated in like manner. After five hours of heating the absorption was 91.5, 86.0, 86.0, 85.0%—av. 87.1%. Further heating did not increase the absorption. Neither did standing at room temperature for two months. When three samples of pure pulegone were so treated 100% absorption was found.

5. Substitution of Alcohol by Dioxane.—Experiment (3) was repeated using dioxane instead of alcohol. Result, 5% absorption.

6. Other Solvents.—Equal volumes of the oil were found completely soluble in propylene glycol, diethylene glycol, propylene glycol laurate and pyridine. It was insoluble in ethylene glycol. Addition of 30% $NaHSO_3$ solution to the glycol mixtures of the

oil caused the oil to separate completely. With propylene glycol laurate a slight emulsion formed. One volume of pyridine is nearly soluble in four volumes of 30% $NaHSO_3$ solution and completely soluble in five volumes. Propylene glycol, its laurate and pyridine were selected for study.

7. Effect of Propylene Glycol and Neutralization.—Three 10-cc. samples of the 1939 oil were treated as in (4) using 10 cc. of propylene glycol. Average of results, 84% absorption.

8. Effect of Propylene Glycol Laurate and Neutralization.—Three 10-cc. samples of the 1939 oil were treated as in (4) using 1 cc. of propylene glycol laurate. Average of results, 30% absorption.

9. Effect of Pyridine.—Three 10-cc. samples of the 1939 oil were each diluted with 10 cc. of pyridine and heated for five hours with 30% $NaHSO_3$ in a cassia flask. Since pyridine is a base, no alkali was needed. Average of results, 86% absorption.

10. Identification of Pulegone.—In each of the above extractions the ketone absorbed was regenerated by separating the aqueous bisulfite portion from the residual oil, making alkaline with Na_2CO_3 and steam distilling. In each case pulegone was the only compound absorbed. It was identified by its odor, taste, boiling point, specific gravity, refractive index, optical rotation, semicarbazone, 2,4-dinitrophenylhydrazone and bisnitroso compound.

It thus appears that assay method (4*b*) is the best. The use of pyridine gives good results but its odor and toxicity are undesirable.

II. COMPARISON OF OILS

The oils from the 1939 and 1940 crops at the University of Florida and 1940 crop from Clewiston are compared in Table I.

Table I.—Comparison of Oils

Properties	1939 Crop, U. of F.	1940 Crop, U. of F.	1940 Crop, Clewiston
Color	Reddish brown	Golden yellow	Golden yellow
Odor	Mint-like	Mint-like	Mint-like
Taste	Mint-like	Mint-like	Mint-like
Sp. gr. $^{20°}_{20°}$	0.9321	0.9326	0.9320
$n^{20°}_{D}$	1.4837	1.4842	1.4845
$[\alpha]^{20°}_{D}$	+23.32	+22.37	+22.21
Pulegone	87.3%	89.3%	90.2%

III. EXAMINATION OF CONSTITUENTS

The oil from the 1940 crop at the University of Florida was examined for non-pulegone constituents. Its physical properties and pulegone content are shown in Table I. By the U. S. P. XI procedure the following were found: A. V., 0.73; S. V., 12.47; E. V., 11.74. The latter corresponds to 4.15% of an alcohol $C_{10}H_{20}O$, calculated as acetate. Acetyl value, 60.6 corresponding to 16.88% of an alcohol $C_{10}H_{20}O$. According to Schmidt (5) pulegone probably forms enol acylates giving an *apparent* acetyl value. Tests for phenols and aldehydes were negative.

Preliminary Treatment.—From 1205 cc. of oil the acids were extracted with Na_2CO_3 solution and set aside for investigation at a future date. Pulegone was next extracted by $NaHSO_3$ according to assay procedure (4*b*). Residual oil was washed with water, dried over anhydrous Na_2SO_4 and filtered. Amount, 125 cc. $n^{25°}_{D}$ 1.4663. One cc. of it yielded a small amount of an unidentified semicarbazone, m. p. 256–7° C., after repeated crystallization from dilute alcohol.

Fractionating Equipment.—The spiral screen packed column and still head described by Lecky and Ewell (6) were built in the Drake Laboratory, University of Florida, and used for this investigation. The washers in the column had an outside diameter of $^{7}/_{16}$ inch and a center diameter of $^{3}/_{32}$ inch, through which a nickel rod was inserted to provide support. Length of packing, 4 feet, 7 turns per inch in Pyrex glass $^{27}/_{64}$ inch diameter. A common type heating jacket was used with two other insulating jackets. Flask and column were electrically heated using transformer controls. Automatic pressure control was used as described by Hershberg and Huntress (7). Efficiency of the column was determined by the method of Morton (8) using a mixture of carbon tetrachloride and benzene. The data of Rosanoff and Easley (9) for composition of gaseous and liquid phases were used. Twenty theoretical plates were found. In fractionating the oil it was found that this column was very sensitive to minute temperature changes, particularly at 5-mm. pressure. This may be due to two factors: (1) fluctuations in the line voltage; (2) the small diameter of column as compared to length. In another investigation the column operated much better at 20-mm. pressure. Voltage regulators or thermostat controls would be an added advantage.

The still head was designed for operation at total reflux with intermittent take-off, so that the distillate was collected in 2-cc. portions. After 18 portions were collected the heat could not be regulated for the reasons explained above and the flooding could not be stopped. The rest of the oil was collected in portions larger than 2 cc. without being able to record the vapor temperature. In view of these facts, it was decided to identify some of the minor constituents present in the first portions obtained. Table II gives the data of the fractionation of 120 cc. of residual oil.

The first vapors were condensed in the cold trap when the pressure was 7.6 mm. and without applying any heat to the distilling flask. This portion was too small for identification. The exact temperature of the last four portions could not be determined due to the flooding in the column. The boiling points were determined by a micro apparatus (10). The specific gravities were determined by using an ampul which held 1.3290 Gm. of distilled water at 25° C.

Limonene.—The properties of fractions 5 and 6 indicated limonene which was established by a tetrabromide derivative, m. p. 101° to 102° C., by Fisher-

Johns melting point apparatus. Fraction 6 did not yield a bromide.

Sylvestrene.—Physical properties of fractions 6 and 8 suggested sylvestrene but it failed to give the sulfuric acid color reaction or a tetrabromide.

Cineol.—Results were negative when an attempt was made to make the resorcinol derivative using fractions 3, 10 and 21.

Table II.—Fractionation of Non-Pulegone Constituents

Fraction	B. P., ° C., 5 Mm.	B. P., ° C., 760 Mm.	Sp. Gr. $\frac{25°}{25°}$	$n_D^{20°}$
1	34.0–34.5	168.0	0.8401	1.4698
2	37.5–38.0	...	0.8391	1.4690
3	38.5–39.0	171.5	0.8374	1.4693
4	39.5–40.0	174.5	0.8440	1.4694
5	39.5–40.0	175.0	0.8442	1.4700
6	42.0–42.5	178.0	0.8519	1.4741
7	42.5–44.0	178.5	....	1.4750
8	47.0–47.5	179.5	0.8601	1.4664
9	51.5–52.0	...	0.8924	1.4590
10	63.5–64.0	204.0	0.8963	1.4570
11	64.0–64.5	203.5	0.8963	1.4575
12	63.0–63.5	205.5	0.8972	1.4550
13	67.0–67.5	...	0.8971	1.4589
14	67.0–67.5	211.0	0.8977	1.4591
15	67.5–68.0	211.0	0.9005	1.4587
16	71.0–71.5	211.5	0.9005	1.4610
17	72.5–73.0	...	0.9040	1.4646
18	73.5–74.0	...	0.9052	1.4669
19	63.0–65.0	...	....	....
20	65.0–72.0	...	....	....
21	67.0–75.0	...	....	....
22	72.0–75.0	...	....	....

Menthone.—This was identified in fraction 12 by means of b. p., sp. gr., $n_D^{20°}$ and its semicarbazone which melted at 183° C. after repeated crystallization from methyl alcohol.

Menthol.—The properties of Fractions 15 and 16 indicated menthol which was substantiated by preparing a phenylurethane, m. p. 107° to 108° C., recorded for *d*- and *l*-menthol 108° to 109° C. The stereoisomerism of the menthol was not determined.

SUMMARY

1. Assay method (4*b*) is recommended for determination of pulegone in the volatile oil of *Pycnanthemum muticum* (Michx.) Pers. which reduced time of assay from approximately 20 or more hours to five hours.

2. Pulegone content and physical properties of three different lots of oil have been determined and found to be very close.

3. Limonene has been identified, not previously reported.

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Derivatives from Hydrogenated Castor Oil

I. 12-Hydroxystearic Acid and Its Alkyl Esters*

By Saul A. Bell† and Abraham Taub‡

Although 12-hydroxystearic acid was first synthesized at the beginning of the century (1) and has been obtainable from hydrogenated castor oil since 1921 (2), little has been done until recently (3) to investigate its pharmaceutical potentialities. Nor has any extensive work been done upon the synthesis and industrial applications of its derivatives. This hydroxy acid, of relatively high melting point and molecular weight, and bearing two functional groups, should provide interesting and useful synthetic compounds. With an annual pro-

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duction in this country of over ninety million pounds of castor oil, and the commercial methods now available for the controlled hydrogenation of this oil, a ready source is provided from which 12-hydroxystearic acid may be obtained easily in high purity and in good yield.

This acid, because it can be prepared practically free from unsaturated acids, is characterized by freedom from color, odor and any tendency toward rancidity. It provides an excellent starting point for the preparation of derivatives having similar characteristics which are so desirable in many pharmaceutical, cosmetic and industrial products. The two classes of derivatives which, it is believed, should prove most productive are those involving modification of the carboxyl and of the hydroxyl groups. In the former are included the esters obtained by combination of the acid with monohydric and polyhydric alcohols; in the latter class are the esters formed with fatty acids, the acid esters of sulfuric, pyrophosphoric and other inorganic acids, and the alkali salts of these acid esters.

In this first paper of the series, the discussion is limited to the normal primary alkyl esters of 12-hydroxystearic acid. The homologous alkyl series C_1 to C_6 and the even-membered derivatives from C_8 to C_{18} have been prepared and characterized. Previous investigators (2, 4, 5, 6, 7, 8, 9) have prepared the lower alkyl esters, C_1 to C_3, and the iso-alkyl esters, C_3 to C_5. But these were made merely as confirmatory derivatives in the identification of the 12-hydroxy acid or as intermediates in its synthesis. No attempt was made to study their properties or field of application.

Such a study of an extended range of this series of esters of a high-melting hydroxylated saturated fatty acid, simulating in many characteristics the pure wax esters, should furnish an insight into the role of the waxes in pharmaceutical ointments, cosmetic creams and industrial products of similar nature, and should aid in the rationalization of formulary practice in these products.

EXPERIMENTAL

Isolation of 12-Hydroxystearic Acid ($CH_3(CH_2)_5CHOH(CH_2)_{10}COOH$).—The method employed in the saponification and isolation of the free fatty acids from hardened castor oil was essentially that suggested by Jakeš and Hökl (10) for the isolation of ricinoleic acid from castor oil to avoid the usual formation of inner esters, polyricinoleic acids and similar side products.

Two hundred grams of powdered (No. 60) hydrogenated castor oil (m. p. 86° C.; hydroxyl value[1] 152.3; saponification number 179.1; iodine number 1.14) were emulsified on a water bath with a solution of 120 Gm. of KOH dissolved in 240 cc. water in a porcelain dish. Heating and stirring were continued for two hours to complete the saponification. The soap was dissolved in one liter of hot water and the fatty acids precipitated by the addition of 400 cc. of diluted HCl (1:1) in a slow stream with constant stirring. The mixture was boiled for 10 minutes to melt the fatty acids and the aqueous layer siphoned off while the acids were still in a liquid state. One liter of boiling water was added, the mixture agitated by boiling for about two minutes and the aqueous layer removed. This washing was repeated until the aqueous layer was free from mineral acid. (About four washings were needed.) The fatty acids were chilled in a refrigerator and the cake coarsely ground in a chilled meat grinder. After drying over night at 50° C. the yield was 187 Gm. (97.4%) melting at 75–77° C.

The fatty acids were extracted continuously for 8 to 12 hours with petroleum ether (boiling range 35–70° C.) to remove acids other than 12-hydroxystearic. The yield of the latter was 149 Gm. (77.7%) melting at 79–80° C., acid value 186.0 (calculated 186.7), iodine value 0.2 (calculated 0). No attempt was made to recover 12-hydroxystearic acid from the petroleum ether extract. The melting point, after two crystallizations from acetone, was constant at 81° C. Previous investigators give melting points as follows: 81–82° C. for an acid obtained by reduction of the HBr addition product of the acetate of ricinoleic acid with zinc or tin and HCl and subsequent saponification with KOH (1); 78° C., 81° C. and 80.5–81° C. for acids obtained by the catalytic hydrogenation of methyl ricinoleate followed by saponification of the ester (4, 5, 8); 75° C. for a crude acid obtained from hydrogenated castor oil (5); 83° C. for an acid obtained from partly reduced castor oil of iodine number 12 (2); 81–82° C. for an acid prepared by the reduction of ricinoleic acid with hydrazine hydrate (9); and 78–79° C. for a completely synthetic optically inactive acid prepared by the Grignard synthesis (7).

Preparation of the Esters of 12-Hydroxystearic Acid.—Two general methods of esterification were used in the preparation of the series. In the esteri-

[1] *Cf. Ind. and Eng. Chem., Anal. Ed.*, 12 (1940), 382.

fication with the normal primary alcohols from C_1 to C_5 the customary procedure was employed: refluxing the acid with a large excess of the appropriate anhydrous alcohol containing 6% to 8% of dry HCl for about eight hours (Method A). For the higher alcohols, where a large excess of alcohol could not be used because of difficulty in its removal and recovery from the ester formed, and for reasons of economy, another method was developed. The latter method required only an equivalent or twofold excess of alcohol and was catalyzed by benzenesulfonic acid in a concentration of 5% of the acid. The reaction medium was benzene, which also served for the entrainment and continuous removal of the water formed in the esterification. A simple distillation trap[1] was used to collect and measure this water (Method B).

Method A.—1. Methyl 12-hydroxystearate: Fifty grams (1/6 mole) of 12-hydroxystearic acid (m. p. 79–80° C.) were esterified by refluxing with 150 Gm. (about 4.5 moles) of 6% methyl alcohol-HCl for eight hours. After standing at room temperature over night, the crystalline product was filtered off on a Büchner funnel and suction continued for several hours. A second crop of ester was obtained by chilling the mother liquor in ice-salt. A yield of 47.1 Gm. (93.6%) of crude ester was obtained, melting at 55° C. (not sharp). No attempt was made to increase the yield by concentration of the mother liquor. After recrystallization from ligroin and acetone, the melting point was constant at 56.5–57.0° C. Saponification number: 181.7 (calculated 178.4); hydroxyl value: 169.5 (calculated 178.4).

2. Ethyl 12-hydroxystearate: Acid was esterified with a twenty-fold equivalent of 6% ethyl alcohol-HCl. Most of the ester was removed by chilling the reaction mixture in ice-salt. The filtrate was diluted with an equal volume of ether and washed successively with dilute potassium carbonate solution and water, and evaporated to dryness to obtain a small second crop of ester. Crude yield, 94.1%. Melting point, after four crystallizations from ligroin, 50.3–51.6° C.

3. n-Propyl 12-hydroxystearate: Esterification was carried out with a tenfold equivalent of anhydrous propyl alcohol (fraction 76–77.3° C.) containing 8% HCl. Crude yield, 86.8%. Melting point, after three crystallizations from ligroin, 48.3–49.5° C.

4. n-Butyl 12-hydroxystearate: A tenfold equivalent of anhydrous *n*-butyl alcohol (b. p. 116–118° C.), containing 10% HCl, was used in the esterification. Crude yield, 87.1%. Melting point, after four crystallizations from ligroin, 43.7–44.9° C.

5. n-Amyl 12-hydroxystearate: After esterification with a tenfold equivalent of anhydrous *n*-amyl alcohol (136°–137° C.) containing 6% HCl and removal of about half the product by chilling in ice-salt, the remainder of ester was obtained by evaporating the mother liquor at reduced pressure on a water bath and chilling over night. Crude yield contained considerable free alcohol. After one crystallization from ligroin the yield was 51.8% melting at 44° C. (not sharp).

Method B.—1. n-Amyl 12-hydroxystearate: Fifty grams (1/6 mole) of 12-hydroxystearic acid were refluxed six hours on an oil bath at about 130° C. with 30 Gm. (1/3 mole) *n*-amyl alcohol and 30 Gm. benzene, using 2.5 Gm. benzenesulfonic acid catalyst. The water formed was removed continuously by means of the trap described, obtaining 2.95 cc. (theoretical = 3.0 cc.). The reaction mixture was washed with dilute potassium carbonate solution and water, chilled in ice-salt and filtered, yielding 25.5 Gm. of ester. The mother liquor was evaporated on a water bath under reduced pressure, leaving a residue of 28 Gm. of impure ester. After one crystallization of the combined material from ligroin the yield was 24 Gm. (39%) melting at 44° C. (not sharp). Further recrystallization of this ester together with that obtained by Method A gave a melting point of 45.0–46.0° C.

2. n-Hexyl 12-hydroxystearate: The acid was esterified with twice the equivalent weight of *n*-hexyl alcohol (b. p. 156–157° C.) refluxing for five hours with the bath at about 130° C. The water separated was 3.1 cc. The reaction mixture was washed with saturated brine solution containing 3% potassium carbonate and then with saturated brine solution. (Later experiments showed that cold water could be used to replace the brine solution.) This was filtered with suction and the benzene and excess alcohol removed on a water bath under reduced pressure. The residue was chilled in an ice-chest over night and filtered with suction. Crude yield was 64 Gm. (100%—contained free alcohol). After two crystallizations from ligroin the melting point was 46.1–47.4° C.

3. n-Octyl 12-hydroxystearate: A 5% excess of *n*-octyl alcohol (b. p. 195–196° C. corr.) was used and refluxed over a period of four hours with the bath temperature at 110–120° C., 3 cc. water separating in that period. The reaction mixture was washed in a separatory funnel with a dilute potassium carbonate solution and then with water. The benzene was removed by warming in a vacuum evaporator on a water bath at about 40 mm. pressure and 60° C. Crude yield was 100% melting at 42–50° C. (Free *n*-octyl alcohol present.) After two crystallizations from acetone the ester melted at 49.5–51.3° C.

4. n-Decyl 12-hydroxystearate: The acid was refluxed during 1.25 hours at 110–120° C. with an equivalent weight of *n*-decyl alcohol (b. p. 121° C. at 16 mm.), obtaining 3.0 cc. of water. The reaction mixture was treated with 5 Gm. potassium carbonate in 200 cc. of water and the benzene removed in a vacuum evaporator. The ester was filtered off with suction and washed twice with water, pressed out on a porous plate and dried over night in a vacuum desiccator. Crude yield was 82.4% melting at 53–

[1] *Cf.* "Methods of A. O. A. C.," 5th Edition, p. 471. The volatile oil separatory trap suitable for oils heavier than water was used.

56° C. After two crystallizations from acetone the melting point was 56–57.2° C.

5. Lauryl ester, n-dodecyl 12-hydroxystearate: An equivalent weight of lauryl alcohol (m. p. 21–23° C.) was used, refluxing about two hours with the bath at about 110° C. and obtaining 3.0 cc. of water. The crude yield was 96.6% melting at 53–59° C. After two crystallizations from acetone the melting point was 60–61.5° C.

6. Myristyl ester, n-tetradecyl 12-hydroxystearate: Myristyl alcohol melting at 38–39° C. was used in equivalent weight, refluxing one hour with the bath temperature at 120–130° C. and obtaining 3.2 cc. water. The crude yield was 95% melting at 60–63° C. After three crystallizations from acetone the ester melted at 61–64° C.

7. Cetyl ester, n-hexadecyl 12-hydroxystearate: An equivalent weight of cetyl alcohol (melting 47–50° C.) was used. Refluxing was continued for 1.25 hours at 115–125° C. collecting 3.2 cc. of water (3.0 cc. were collected in one hour). The crude ester contained some NaCl from the saturated brine solution used in washing as well as a considerable amount of organic material insoluble in acetone and melting above 100° C. This latter was formed in all other cases where the esterification was continued beyond the point when the theoretical 3 cc. of water had separated. After two crystallizations from acetone the ester melted at 66.5–69.5° C.

8. Stearyl ester, n-octadecyl 12-hydroxystearate: The stearyl alcohol (melting at about 58° C.) was used in equivalent weight and the mixture refluxed 1.5 hours during which time 3.0 cc. of water separated. A yield of crude ester (82.8%) was obtained melting at 74–75.5° C. After one crystallization from acetone the melting point was 76–76.5° C.

PROPERTIES AND APPLICATIONS

Melting Points and Solubilities.—In the preliminary evaluation of these compounds for pharmaceutical, cosmetic and industrial uses, their melting points and their solubilities in the commonly used solvents were determined. These are summarized in Tables I and II.

Table I.—Melting Points of the Esters of 12-Hydroxystearic Acid

Ester	Mol. Wt.[a]	Hydroxyl Value[a]	M. P., ° C.
Methyl	314.5	178.4	56.6–57.0
Ethyl	328.5	170.8	50.3–51.6
n-Propyl	342.5	163.8	48.3–49.5
n-Butyl	356.6	157.3	43.7–44.9
n-Amyl	370.6	151.4	45.0–46.0
n-Hexyl	384.6	145.9	46.1–47.4
n-Octyl	412.7	136.0	49.5–51.3
n-Decyl	440.7	127.3	56.0–57.2
n-Dodecyl (lauryl)	468.8	119.7	60.0–61.5
n-Tetradecyl (myristyl)	496.8	112.9	61.0–64.0
n-Hexadecyl (cetyl)	524.9	106.9	66.5–69.5
n-Octadecyl (stearyl)	552.9	101.5	76.0–76.5
12-Hydroxystearic acid	300.5	186.7	81.0
Hydrogenated castor oil		152.3[b]	86.0

[a] Calculated.
[b] Found.

The melting points of the esters appear to follow a fairly smooth curve of the pattern usually found in such a series with the minimum occurring at the butyl ester. From these data it appears that the solubility in this series of esters is a function of the melting point rather than of the molecular weight—the butyl ester, which has the lowest melting point, being in most cases the most soluble. All the esters were found to be very soluble in the hot (about 55° C.) solvents, the cetyl and stearyl esters being slightly less soluble than the others. Hot solutions of the higher esters in toluene and the oils upon cooling formed mixtures which had the appearance of gels but which were not the true gels that form from solutions of the hydroxy acid or of the hydrogenated castor oil.

Some miscibilities of the melted esters and hydroxy acid with selected pharmaceutical and industrial materials were determined.

Table II.—Comparative Solubilities of the Esters of 12-Hydroxystearic Acid at 25° C.

	Cc. of Solvent Needed to Dissolve 1 Gm. of Ester					
Ester	Ethanol	Acetone	Ligroin	Toluene	Mineral Oil	Cottonseed Oil
C_1	3	5	20	2	..	20
C_2	2	3	10	1	40	20
C_3	2	3	4	1	40	20
C_4	1	1	2	1	30	10
C_5	1	2	2	1	30	20
C_6	2	3	2	1	30	20
C_8	10	5	5	2	50	30
C_{10}	50	20	20	3	..	40
C_{12}	150	50	50	5	..	50
C_{14}	200	80	50	5	..	50
C_{16}	...	450	200	20	..	..
C_{18}	...	...	450	50	..	..
Acid	5	20		200		
Oil	200	200		150		

It was found that Peru balsam was only incompletely miscible with these compounds at their respective melting points, and similarly ichthammol was only slightly miscible. However, the hydroxy acid appeared to react chemically with the ichthammol and formed a homogeneous mixture in all proportions. Tetrabromofluorescein, a representative of a group of certified cosmetic colors, was only poorly soluble in the melted esters although the latter were definitely tinted. On the other hand, both crude oil and coal tar were miscible in all proportions. Carbitol was miscible in all proportions with the hydroxy acid and with the melted esters, C_1 to C_4, somewhat less miscible with those from C_5 to C_{12}, and from 10 to 20 volumes of solvent were needed to form a clear solution of the esters of the C_{14} to C_{18} alcohols. The esters were less miscible in propylene glycol, the extent of miscibility being a function of the molecular weight of the esters rather than of their melting point. The acid and the methyl ester were miscible in all proportions while the butyl ester, although completely miscible with an equal weight of glycol at 75° C., was only partially miscible at its melting point and required 20 volumes of glycol to make a clear solution at that temperature. The stearyl ester was immiscible with up to 50 volumes of propylene glycol. All the members of the series including the hydroxy acid were found to be immiscible with glycerine but miscible in all proportions with petrolatum and with lanolin.

According to a recent research on commonly used natural waxes (11), a comparison of the chemical compositions of these waxes shows that, in general, they consist of similar components; in fact, beeswax, carnauba, candelilla and montan contain compounds with identical composition, *i. e.*, acids, alcohols and hydrocarbons with 24 to 34 carbon atoms. The characteristic properties of these waxes which determine their individual applicability to specific uses result from variation in proportions of acids, alcohols and hydrocarbons, differences in chain length of the two components of the ester, and the presence of small amounts of additional distinctive components such as resin-like substances and ketones. It appears probable that, without any attempt at actual duplication of composition, waxes can be made to definite specifications by rational combinations of such a series of esters as have been prepared, both with each other and with appropriate acids, alcohols and hydrocarbons. Such products would have the desirable absence of color, odor and tendency toward rancidity as well as predetermined definite and permanent compositions.

The characteristics of various combinations of the esters of 12-hydroxystearic acid were determined, using fused mixtures of equal parts of two of the esters. After refrigeration for 12 hours, the mixtures were examined to ascertain the nature of their structure, the type of fracture and the melting point. The results are summarized in Table III.

No broad generalizations can be made from this table, but it appears that unless the two components differ from each other by at least two carbon atoms the mixture retains the crystalline structure of the origi-

Table III.—Characteristics of Ester Mixtures

Mixture	Structure	Fracture	M. P., ° C.[a]
Methyl-ethyl	Crystalline	Crystalline	48.3
Methyl-propyl	Semi-crystalline	Crystalline	44.0
Methyl-decyl	Waxy	Conchoidal	44.0
Ethyl-propyl	Crystalline	Crystalline	47.4
Propyl-butyl	Crystalline	Crystalline	43.0
Propyl-amyl	Waxy	Conchoidal	39.2
Butyl-amyl	Crystalline	Crystalline	42.0
Butyl-hexyl	Waxy	Conchoidal	37.5
Butyl-decyl	Waxy	Conchoidal	42.0
Octyl-decyl	Waxy	Conchoidal	47.3
Decyl-lauryl	Waxy	Conchoidal	52.4
Lauryl-myristyl	Waxy	Conchoidal	56.0
Myristyl-cetyl	Semi-crystalline	Crystalline	60.3
Cetyl-stearyl	Crystalline	Crystalline	73.0

[a] Determined by the U. S. P. method for materials belonging to Class II. ("United States Pharmacopœia XI," p. 455.)

nal pure esters. Even in such cases, if the esters are too near the extremes of the series, the resultant mixture is not amorphous as is indicated in the methyl-propyl, myristyl-cetyl and cetyl-stearyl pairs, which remain more or less crystalline. The most satisfactory pairs were the decyl-lauryl and lauryl-myristyl, although others, as indicated, had the amorphous wax-like structure and brittle conchoidal fracture sought for. It was further found that addition of 10% of free alcohol or 10% of hydroxy acid or 5% of each to the higher esters produced crystalline mixtures of slightly lower melting point while similar additions to amorphous ester pairs did not alter their waxy structure.

It appears likely that esters of 12-hydroxystearic acid prepared with some of the standard mixtures of homologous higher alcohols (saturated *n*-primary) commercially available would have the desired wax-like character without further admixture.

Ointments and Cosmetic Creams.—Mixtures of white petrolatum containing 10% of the ester as a hardener were prepared by fusion with each of the esters in the series. All the esters as well as the hydroxy acid were completely compatible with the petrolatum, forming homogeneous mixtures and imparting a degree of hardness depending on the melting point of the ester. It is obvious, therefore, that these esters permit a degree of flexibility in the compounding of ointments. For example, by use of a high-melting ester greater amounts of liquids such as pine tar may be incorporated without producing too soft a product.

The esters *per se* were not suitable in place of beeswax as hardeners in beeswax-borax type cosmetic creams. Preliminary attempts to induce emulsification in this type of cream by the addition of 10% of free 12-hydroxystearic acid were unsuccessful. On the other hand, tri-isopropanolamine produced satisfactory cosmetic creams. The two formulas which follow gave creams of good appearance, consistency and stability.

COLD CREAM (ALMOND OIL TYPE)

Ester + 10% acid	24.5 Gm.
Almond oil	56.0
Tri-isopropanolamine	1.2
Water	18.3

COLD CREAM (MINERAL OIL TYPE)

Ester + 10% acid	16.2 Gm.
Mineral oil	58.0
Tri-isopropanolamine	0.8
Water	25.0

The creams were made by adding the tri-isopropanolamine dissolved in the water to the melted ester, acid and oil,with vigorous mechanical stirring. After emulsification occurred, the cream was stirred occasionally until cold and congealed. It was found that both the low-melting and high-melting esters gave creams of poor quality with these formulas, the former because they did not impart sufficient hardness and the latter because they formed granular creams. The C_8 to C_{12} esters were satisfactory, the decyl ester proving the best. A combination of two esters melting in this middle range (*e. g.*, lauryl-myristyl) was even better. Increasing the amount of ester made a somewhat stiffer but not a better cream. Increasing the amount of free acid with the ester to 20% made no improvement unless the amount of tri-isopropanolamine was correspondingly increased.

Emulsified cosmetic creams of the water-miscible type were prepared using Aerosol OT as the emulsifying agent according to the following formula:

Cetyl ester	20 Gm.
Mineral oil	40
Water	20
Aerosol OT (10% aqueous)	20

The melted ester and oil were added to the Aerosol OT in water with vigorous stirring until emulsification occurred and the cream stirred occasionally until cooled and congealed. This type of cream had by far the finest texture and properties. By introducing free cetyl alcohol and free 12-hydroxystearic acid, the appearance of the finished cream was varied as follows: Addition of 10% cetyl alcohol to the cetyl ester (*viz.*, 2% of the total weight) produced a high luster cream; addition of 10% 12-hydroxystearic acid to the cetyl ester produced a matte cream of harder consistency; addition of 5% each of alcohol and acid produced a cream of medium luster and medium consistency. These creams proved very stable but permitted the addition of

only limited quantities of 10% solutions of aluminum chloride or of tannic acid without being materially altered. This limitation in the addition of electrolytes and acidic substances is due more to the characteristics of the emulsifier than to the ester used. Because of the neutral character of the esters, incompatibility with dilute acids, alkalies and electrolytes should not be encountered.

Physical limitation precludes the preparation of the large number of possible combinations of the 12-hydroxystearic acid esters in varying proportions with related fatty alcohols and acids. But the examples cited should suffice to show the adaptability of these esters to formulations involving wax-type components. The desirable qualities of these esters invite further studies of their pharmaceutical and industrial applications. The problem of making synthetic products which approximate or even improve upon the natural waxes is indeed a complex one, but it is believed that a satisfactory approach has been made through the synthesis of these esters of 12-hydroxystearic acid.

SUMMARY

Methods have been given for the isolation of 12-hydroxystearic acid from commercial hydrogenated castor oil, and for the syntheses of a series of twelve of its esters with normal primary alcohols. A number of their physical properties have been evaluated and some of their applications to ointments and similar bases have been suggested. The results indicate the possibilities of utilizing these esters in the formulation of waxes having reproducible properties and whose qualities should prove superior to the natural waxes in respect to stability and freedom from color, odor and rancidity.

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A Phytochemical and Pharmacological Study of the Berries of *Phytolacca americana* Linné (Fam. Phytolaccaceae)

By Laurine D. Jack and Charles H. Rogers†*

The berries of *Phytolacca americana* (*decandra*) have been known in clinical medicine for many years. Phytochemical and pharmacological investigations have consisted of a few incomplete reports. The present work was undertaken with the purpose of making a more complete study.

* Abstracted in part from a thesis submitted by Laurine D. Jack to the Graduate Faculty of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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EXPERIMENTAL

A. PHYTOCHEMICAL INVESTIGATION

Preliminary Study.—Moisture Determination: The moisture content was determined by the volatile immiscible solvent method of the United States

Pharmacopœia XI (4). Moisture was present to the extent of 6.5 per cent.

Ash Determination: The total ash was found to be 4.22 per cent, of which 1.78 per cent was water insoluble and 2.45 per cent was water soluble. The alkalinity of the water insoluble ash was 1.41. The amount of acid insoluble ash was 1.05 per cent.

Extraction with Various Solvents: In order to ascertain the general character of the constituents of Phytolacca Berries 40 Gm. of the drug were extracted in a Soxhlet apparatus with the following solvents: petroleum ether, ethyl ether, chloroform, ethyl alcohol, and water. After the soluble constituents had been completely extracted with each solvent, the latter was removed by evaporation. The residual extracts were dried over sulfuric acid at room temperature until constant weight resulted. Petroleum ether extracted 7.87 per cent; ethyl ether, 0.51 per cent; chloroform, 0.84 per cent; ethyl alcohol, 26.19 per cent; and water, 10.79 per cent.

Test for Alkaloid: Ten grams of the drug were macerated with prolius fluid for several days after which time the fluid was decanted from the drug. It was shaken in a separatory funnel with 2 per cent sulfuric acid. The acid extract was tested for alkaloids with the usual alkaloidal reagents. All tests were negative.

The drug (100 Gm.) was examined by the Stas-Otto method (2) in order to test for alkaloids. The residues which were tested with the alkaloidal reagents yielded negative results.

Large Scale Extraction.—Fifty pounds of the drug (No. 20 powder) were extracted in a Barnstead Extractor with ethyl alcohol until the drug was exhausted. Most of the alcohol was removed from the extract by distillation. The semi-solid residue was incorporated with a sufficient amount of dried marc and was packed in a percolator. It was then extracted with petroleum ether.

Investigation of Petroleum Ether Extract.—The residue from the petroleum ether extract was a green oil. After standing for a few days, minute crystals separated from the oil.

Investigation of Crystals: Microscopically the crystals appeared both as rosettes and as platelets. They were very soluble in petroleum ether and in ethyl alcohol. The melting point range of the purified crystals was 296° C. to 297° C. The specific rotation was +14° at 20° C. Qualitative analysis indicated the absence of nitrogen, sulfur and halogens. The Liebermann-Burchard test for sterols resulted in a permanent red color. A chloroformic solution of the substance decolorized a bromine solution. The molecular weight as determined according to the Rast method was 443.9.

Analysis: Calcd. for $C_{28}H_{46}O_3$: C, 78.07; H, 10.77. Found: C, 77.47; H, 10.62.

The crystals (0.3838 Gm.) were dissolved in 30 cc. of amyl alcohol, 10 cc. of 15 per cent hydrochloric acid were added, and a sufficient amount of ethyl alcohol to form a homogeneous mixture. The solution was heated for 6 hours under a reflux condenser. The alcohol was removed by steam distillation and the product was recovered from the aqueous solution with ethyl ether. The crystals melted at 286.5° C. to 288° C.

Analysis: Calcd. for $C_{27}H_{44}O_3$: C, 77.81; H, 10.65. Found: C, 78.02; H, 10.68.

A portion of the crystals (0.1 Gm.) were acetylated with a mixture consisting of 10 cc. of acetyl chloride and 5 cc. of pyridine. The crystalline product obtained melted at 303° C. to 304° C. The molecular weight as calculated upon the basis of the acetyl number was found to be 468.7.

Analysis: Calcd. for $C_{30}H_{46}O_4$: C, 76.21; H, 10.24. Found: C, 76.36; H, 10.41.

The acetyl number and the combustion indicated that one acetyl group had been introduced into the molecule. It was concluded that one oxygen atom existed as a free hydroxyl group.

According to the alkoxy determination, no alkoxy groups were present. An attempt was made to reduce the compound catalytically at room temperature, but no reduction occurred. Chromic acid oxidation yielded a residue which apparently was a mixture, for all attempts to crystallize it failed.

Determination of Oil Constants: The oil obtained from the petroleum ether extract was decolorized with charcoal and the following constants were determined: Specific gravity $\frac{25°}{25°}$, 0.89937; index of refraction, $N_D^{20°}$, 1.47046; specific rotation, $[\alpha]_D^{26°}$, +0.28°; acid value, 15.76; saponification value, 189.13; ester number, 173.37; iodine value, 105.22.

Saponification of Oil: The oil was saponified with alcoholic potash. A dilute aqueous solution of the saponified product was prepared and extracted with several portions of ethyl ether in order to remove the unsaponifiable material. From the aqueous solution the free fatty acids were liberated upon the addition of a diluted mineral acid. The resultant aqueous solution was evaporated to dryness.

Detection of Glycerol: The above residue was extracted with absolute alcohol. The residue, which was obtained after evaporation of the alcohol, was dissolved in a small amount of water, a few crystals of potassium bisulfate were added, and the mixture was heated. The odor of acrolein was perceptible which indicated the presence of glycerol.

Isolation of Free Fatty Acids: The combined fatty acids were separated into the saturated and unsaturated fractions according to the lead-alcohol precipitation method of Twitchell (3). About 12 per cent of the fatty acids were saturated.

The methyl esters of the saturated fatty acids were prepared and fractionally distilled *in vacuo* at 4 mm. Hg. Each fraction was saponified and the free fatty acids were liberated upon the addition of a diluted mineral acid. The melting point range

and the mean molecular weight of each fraction was determined. Assuming that no fraction contained more than two adjacent homologs the percentages of the acids were calculated. Accordingly, the mixture of saturated fatty acids consisted of: Palmitic acid, 50.7 per cent; stearic acid, 45.6 per cent; arachidic acid, 3.7 per cent. Qualitative tests for palmitic and stearic acids were positive. The qualitative test for arachidic acid was negative.

The unsaturated fatty acids were methylated and distilled in the same manner as previously described. The iodine value for each fraction was determined. Each fraction was reduced catalytically at room temperature; ethyl alcohol was used as the solvent and platinum black as the catalyst. The residues were saponified and crystals were obtained. The melting points and saponification values of the crystals were determined. The molecular weights were calculated. The values corresponded to those of stearic acid. The *p*-brom phenacyl ester of each crystalline fraction was prepared. Mixed melting points with Eastman *p*-brom phenacyl ester of stearic acid showed no depression. It was concluded that the reduced acid in each case was stearic acid. A portion of the unsaturated acids were brominated. No hexa- or tetrabrom derivatives were prepared so it was believed that the eighteen carbon unsaturated acid was oleic acid.

Unsaponifiable Material: The unsaponifiable material consisted of two crystalline products which were impregnated with an oily substance. The latter was removed inasmuch as it was more soluble in petroleum ether than the crystalline portion. The crystalline products were separated by fractional crystallization from ethyl alcohol and finally from isopropyl ether. According to the Liebermann-Burchard test one of the products was a sterol and the other product was later identified as Hentriacontane.

Investigation of Sterol: The crystals melted at 166° C. Qualitative analysis indicated the absence of nitrogen, sulfur and the halogens. It was believed that the compound was a sterol because the Liebermann-Burchard, Rosenheim and Salkowski tests for sterol were positive. A chloroformic solution of the crystals absorbed bromine.

Analysis: Calcd. for $C_{31}H_{56}O.H_2O$: C, 80.44; H, 12.64.

Found: C, 80.54; H, 12.54.

The crystals were acetylated with acetic anhydride. The melting point of the acetylated product was 175° C. to 175.5° C. The acetyl determination indicated that one acetyl group had been introduced into the molecule. The molecular weight was calculated to be 484.7.

Analysis: Calcd. for $C_{33}H_{58}O_2$: C, 81.40; H, 12.01.

Found: C, 81.13; H, 10.96.

Investigation of Hentriacontane: The melting point of the crystalline product was 67.5° C. Qualitative analysis indicated the absence of nitrogen, sulfur and the halogens. It was insoluble in hot concentrated sulfuric acid. A chloroformic solution did not absorb bromine. The molecular weight was determined by the Rast method and was found to be 432.2. The crystals were optically inactive.

Analysis: Calcd. for $C_{31}H_{64}$: C, 85.24; H, 14.77.

Found: C, 84.24; H, 14.87.

Extraction of Pigment.—The whole berries of *Phytolacca americana* which were obtained from the University of Minnesota Medicinal Plant Gardens were extracted with cold glacial acetic acid. Upon the addition of ethyl ether a red gummy substance was precipitated. It was very soluble in water. Its color reactions with acids, alkalies and lead acetate place it in the same class as the anthocyanins of unboiled beet juice and the Chenopodiaceæ.

B. PHARMACOLOGICAL INVESTIGATION

In carrying out the pharmacological investigation the principal motive was to ascertain the relative toxicity of the berries of *Phytolacca americana*. For this purpose two fluidextracts were prepared—one containing 50 per cent of alcohol by volume and the other containing 25 per cent of alcohol by volume. Also an infusion and a colloidal solution were used. The latter represented a fraction of the alcoholic extract.

Effect on Frogs.—For this purpose the fluidextract containing 50 per cent of alcohol was used and when injected into frogs *via* the ventral lymph sac, a depression was produced. Likewise, a depression occurred in the control frog. One of the frogs which received the fluidextract died, whereas the control which received an injection consisting of a corresponding amount of alcohol survived. It was believed that there might be something in addition to the alcohol which was producing a depressant effect upon the frog.

Effect on Rats.—When intraperitoneal injections of the fluidextract (50 per cent alcohol) were made into rats, a deep degree of narcosis resulted. This also occurred in the control, however the effect was not so pronounced. In the rats which had received the drug it was noted that the reflexes were largely absent, whereas in the control they were present but the reflex time was prolonged. The drug caused a decrease and an irregularity in the respiratory rate. One rat died as a result of respiratory paralysis. It was concluded that the toxic effect of the drug was of the nature of a narcotic or depressant which was more intense than that caused by alcohol.

Effect on Rabbits.—Intraperitoneal injections of the fluidextract (25 per cent alcohol) into rabbits had no effect. The reflex time was normal and there was no indication of any paralysis.

The fluidextract (25 per cent alcohol) was administered orally to rabbits by means of a stomach tube. No abnormal effects were produced.

Several experiments were conducted in which the drug in the form of the fluidextract, infusion or colloidal solution was administered intravenously and the blood pressure was recorded simultaneously. All of these experiments indicated that the drug caused a general depression. The blood pressure fell after each injection of drug but it was not sustained at the lower level. It rose almost as quickly as it fell. However, it never reached its preceding value. Therefore, throughout the course of the experiment there was a gradual decrease in the blood pressure. In general the carotid pulse rate decreased throughout the experiment. Respirations appeared to be normal until the fatal injection was approached. Eight deaths were due to respiratory failure and two deaths were due to cardiac paralysis. For the sake of comparison, two experiments were carried out in a similar manner in which Fluidextract of Phytolacca Root was used. One death was due to respiratory failure and the other was due to cardiac paralysis. These experiments indicated that Phytolacca Root was five to eight times as active as Phytolacca Berries.

It was believed that the action of the drug was primarily upon the medullary centers. The heart rate in some cases was quite irregular and in a few experiments it was believed that extra systoles had occurred. In the majority of the experiments, as fatality was approaching the rate of respiration decreased and the pressure waves were shallow, which indicated either a decreased demand for oxygen or lack of the normal gas exchange. In several of the experiments it was noted that the pulse pressure increased. This was due to a decrease in the diastolic pressure. Such a condition might be indicative of a failing circulation.

Effect on Cats.—Intraperitoneal injections were made into cats. There was no immediate indication of any toxicity; however, a slow death occurred. Goldstein, Jenkins and Thompson (1) had observed under the same conditions with Fluidextract of Phytolacca Root that an ascending paralysis had occurred.

When intravenous injections of the fluidextract (25 per cent alcohol) were made into a cat the same effect was produced as with rabbits.

SUMMARY

From the berries of *Phytolacca americana* the following constituents were isolated:

1. Glycerol as the glyceryl ester of naturally occurring fatty acids.
2. Saturated Fatty Acids.
3. Oleic Acid.
4. A Sterol.
5. A Compound which resembled a Sterol.
6. Hentriacontane.

The pharmacological experiments showed that:

1. The Fluidextract of Phytolacca Berries produced a mild degree of depression.
2. The toxicity of Phytolacca Berries was not as great as that of Phytolacca Root.

From the pharmacological data it can be concluded that Phytolacca Berries have no advantage over Phytolacca Root.

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Tabasco a Substitute for Capsicum*

By Carmel H. Olden and E. V. Lynn†

In a recent number of the Journal, Youngken (1) called attention to the pungency of tabasco peppers, the fruit of *Capsicum anuum* L. var. *conoides* Irish. Preliminary tests by us showed that the material is at least as pungent as capsicum, and we decided to determine by extraction whether it could advantageously be employed as a substitute and whether it actually contains capsaicine. We had available also a quantity of waste from making tabasco sauce and used this for extraction. Only the pulpy part of the fruit is employed in making sauce, the waste being largely seeds and cortical tissue.

EXPERIMENTAL

The material was ground to a coarse powder and extracted according to Tice (2) with ether. It

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was necessary to carry out the process long after the extract was colored, since the removal of pungency is slow and does not parallel that of color. From 4 Kg. of waste was obtained about 800 cc. of oleoresin. This was mixed with twice its volume of heavy liquid petrolatum and extracted three times, using 1500 cc. of 57 per cent alcohol each time. The alcohol was removed by distillation, the aqueous residue was completely extracted by ether, and the solvent was removed again by distillation. The residual crude substance was mixed with 25 Gm. of purified lithium hydroxide and 1500 cc. of water, and the whole was boiled for about 10 minutes with vigorous stirring. After this had stood for 24 hours and had been filtered, carbon dioxide was run in to saturation, giving a fine, crystalline precipitate. This was dried in a desiccator and crystallized repeatedly from hot petroleum benzin, in which it is only slightly soluble. The total yield of highly purified crystals was 8.81 Gm. or 0.22 per cent. It is estimated that the total amount present in the tabasco waste is about 0.4 per cent.

The crystalline product was completely identified as capsaicine. It gave the color reaction with ammonium vanadate or vanadium oxychloride in acetone. The corrected melting point was found to be 64.8°. When it was intimately mixed with an equal amount of the purified substance from capsicum which melted at the same temperature, this mixture was found to have the identical melting point.

Tests with smaller amounts of material indicated that the whole tabasco pepper contains about 0.4 per cent of capsaicine. We conclude that the latter is distributed uniformly throughout the fruit and that the waste is just as pungent as the whole peppers. This was confirmed by organoleptic tests, which also indicated that either contains about four times as much pungency as Capsicum U. S. P. In the literature the latter is said to yield 0.007 to 1.0 per cent, but actually no one has apparently made an accurate determination; the most reliable statements place the content at about 0.1 per cent. In any event, tabasco peppers or any portion of them contains at least as much and probably several times this.

CONCLUSION

The recommendation is accordingly made herewith that the domestic tabasco pepper be considered for inclusion in the Pharmacopœia in place of capsicum, or at least that the monograph be revised to include tabasco pepper.

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The Assay for Menthol*

By Charles O. Wilson†

It has been the experience of the author that the United States Pharmacopœia XI assay for menthol in oil of peppermint gave very faulty results and according to the literature, other workers have also found this to be true. Since the method now used was first introduced by Power and Kleber (1) there has been a continued effort to remedy some of its more obvious sources of error. This subject has been under investigation by the author for some time and a report was practically completed when Brignall (2) published his work on oil of peppermint, covering the same method of approach. The evidence here offered, therefore, serves to confirm Brignall's findings.

Elliott (3) has pointed out the difficulty of completely removing the excess acetic anhydride from the acetylized oil. He suggested that one add 10 cc. of water to the acetylized oil and boil the mixture for ten or fifteen minutes to break up the acetic anhydride, then wash several times with dilute sodium carbonate solution to remove the acetic acid. Nelson (4) states that the reagents used should be of the best quality and that the sodium acetate must be perfectly dry for best results. In 1939 Baldinger (5) carried out experiments on oil of peppermint, varying the time of both acetylation and saponification. The saponification time for most consistent results was found to be forty-five to sixty minutes. He also found that the acetylation time may vary greatly, that in some cases fifteen minutes was sufficient. The author has

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also obtained satisfactory results when acetylation time has been only fifteen or twenty minutes. A method suggested by Regilyant (6), using one per cent phosphoric acid as a catalyst in acetic anhydride was tried and gave good results when acetylation time was only ten minutes.

Considerable trouble has been encountered in the estimation of the end-point after saponification, both for ester content and total menthol. The practice now in use, using phenolphthalein T.S., U. S. P. XI as indicator, requires the titration to be carried out until no color remains in the mixture. As stated by Baldinger (5), checks of one per cent are considered satisfactory by some volatile oil companies for the menthol assay. To overcome this difficulty, Nelson (4) suggested adding thirty to forty drops of phenolphthalein as indicator. Both he and Baumgarten (7) recommended diluting with water prior to titration and indicated very satisfactory results. Baumgarten (7) tried methylene blue as an indicator but states that dilution with water was better. The following procedure for the estimation of ester content in oil of peppermint involves dilution with water.

EXPERIMENTAL

Samples of oil were assayed for ester content using the U. S. P. XI method and the modified method suggested here.

The oil was weighed and saponified in the usual manner, but after refluxing and partially cooling, the mixture was diluted with 50 cc. of distilled water, allowed to stand for about five minutes and then titrated with 0.5*N* sulfuric acid, using two or three drops of phenolphthalein T.S. as indicator. Table I shows the comparison of the U. S. P. XI and the modified method. If allowed to stand a few minutes after the addition of water, the mixture tends to clear up and the red coloration is less noticeable.

Table I

Sample	U. S. P. XI, Per Cent Esters	Modified U. S. P. XI, Per Cent Esters
1*a*	7.0	7.1
1*b*	6.9	7.1
1*c*	7.0	7.1
2*a*	10.0	10.3
2*b*	10.1	10.2
2*c*	10.0	10.2

The end-point, using the U. S. P. XI method, was very difficult to distinguish and in some cases it was practically impossible to notice a definite change of color. Results expressed in Table I show that the addition of water (4), (7) does affect the results, as an average of 0.16 per cent more esters are found by its use. It is thought that these results are very satisfactory, as only one-third of the indicator is required and the end-point is very sharp and is easily observed.

In past years, Verley and Bolsing (8), Freed and Wynne (9), Delaby, *et al.* (10), and Rabaté (11) have used acetylizing mixtures of acetic anhydride and pyridine to determine primary and secondary alcohols. The following method is based on their findings.

In a tared 125-cc. Erlenmeyer flask weigh accurately about 2 Gm. of oil of peppermint. Add 5 cc., accurately measured, of acetylizing agent (one part acetic anhydride and four parts of anhydrous pyridine) and reflux in boiling water for thirty minutes, using a water-cooled condenser. The pyridine is made sufficiently anhydrous by distilling and collecting that portion of the distillate coming over between 114° and 116° C. Through the condenser add about 30 cc. of warmed distilled water and continue heating for fifteen minutes. This causes the complete transformation of the excess acetic anhydride to acetic acid. Allow the system to cool and before disconnecting the Erlenmeyer flask rinse the condenser once with 15 cc. of distilled water. Care should be exercised to keep the flask stoppered to prevent loss of any volatile acetic acid. The contents of the flask are now titrated with 0.5*N* alcoholic potassium hydroxide, using two drops of phenolphthalein T.S., U. S. P. XI as an indicator. The end-point is very sharp.

A blank determination must also be carried out and the number of cubic centimeters of 0.5*N* alcoholic potassium hydroxide required to neutralize the acetic acid produced by 5 cc. of the acetylizing agent noted.

The number of cubic centimeters of alcoholic potassium hydroxide required for the blank, minus the number of cubic centimeters required for the sample, multiplied by 0.0781,[1] equals the number of grams of free menthol present. The percentage of total menthol may be obtained by multiplying the percentage of esters by 0.7880 and adding to the percentage of free menthol.

The acetylizing agent was found to be stable for about one week, but after that time low results were obtained by its use. After two weeks, only results of 93 per cent or less were obtained. Light causes a darkening of the reagent, so it must be stored in a dark place. Pyridine, after being

[1] One gram of menthol requires 0.6535 Gm. of acetic anhydride in reacting to form one molecule of menthyl acetate and one molecule of acetic acid. The acetic acid formed is 0.3844 Gm. and is equal to 12.8 cc. of 0.5*N* alcoholic potassium hydroxide. Therefore, 1 Gm. of menthol, divided by 12.8, equals 0.0781 Gm. of menthol per cc. of 0.5*N* alcoholic potassium hydroxide.

rendered anhydrous, is suitable for use after several months, if kept protected from light and tightly stoppered.

Samples of oil of peppermint were assayed for menthol by the U. S. P. XI and the suggested method. Results are in Table II.

Table II

Sample	Per Cent Esters, Modified Method	U. S. P. XI, Total Menthol, Per Cent	U. S. P. XI, Free Menthol, Per Cent	Suggested Method, Per Cent Free Menthol
1	7.1	52.1	46.5	43.6
2	10.2	52.0	44.0	41.0
3	5.2	50.2	46.1	43.3
4	5.6	51.6	47.2	44.7
5	7.3	52.3	46.6	43.8
6	6.1	50.5	45.7	42.5

The data show that the suggested method gave an average free menthol content of 2.8 per cent lower than that of the U. S. P. XI method. A cause for this difference is partially explained in Tables III, IV and V.

The minimum requirement of the U. S. P. XI for oil of peppermint is 50 per cent total menthol. Brignall (2) found 43.0 per cent free menthol by his method equivalent to the U. S. P. XI requirement, while in the author's work 43.3 per cent of free menthol was found.

Solutions of purified menthol in rectified oil of turpentine U. S. P. XI were used to test the accuracy of this method as compared with that of the one now in the U. S. P. XI. Blank determinations by the U. S. P. XI method on the rectified oil of turpentine gave average findings of 0.3 per cent esters, calculated as menthyl acetate, 1.2 per cent total alcohols calculated as menthol, and 1.0 per cent free menthol. No free alcohols were found in rectified oil of turpentine by the suggested method. In Table III are recorded the findings of method comparison, taking into account the alcohols found in the controls.

Table III

Sample	Calculated Per Cent Menthol	U. S. P. XI Method, Per Cent Total Menthol Minus 1.2%	Per Cent Free Menthol Minus 1.0%	Suggested Method, Per Cent of Menthol
1	8.1	9.1	8.9	8.0
2	17.7	18.8	18.6	18.1
3	34.2	35.8	35.6	34.4
4	69.3	70.5	70.3	69.1
5	85.1	86.3	86.1	85.3

The data in Table III indicate that the suggested method gives better determinations than the U. S. P. XI procedure. The percentages of menthol found by the two methods do not differ to the same extent as they did in Table II. It appears then that some potassium hydroxide is consumed in other than ester saponification (5) and/or other constituents of the oil are acetylized by the official procedure (Table IV) and/or small errors are present, causing high results, due to the manipulations in the U. S. P. XI procedure and, as noted by Table V, the suggested method is more exact than that of the U. S. P. XI.

The fact that by the U. S. P. XI method 1.2 per cent total alcohols are found in rectified oil of turpentine and none are accounted for by the suggested method indicates that some non-alcoholic constituents are acetylized by the U. S. P. XI procedure. Experiments were carried out to compare the two methods of acetylization, that of the U. S. P. XI, using acetic anhydride and anhydrous sodium acetate and that of the suggested method, using acetic anhydride and pyridine. Oil of peppermint was assayed for total menthol, following the U. S. P. XI procedure but differing only in the method of acetylizing the oil. Table IV expresses the results found.

Table IV

Sample	Per Cent of Esters	Per Cent Total Menthol, U. S. P. XI	Acetic Anhydride and Pyridine, Per Cent Total Menthol, U. S. P. XI
1	7.1	52.1	50.0
2	10.2	52.0	49.5
3	5.2	50.2	47.8
4	5.6	51.6	48.5
5	7.3	52.3	50.1

It does appear, then, by the lower percentage of menthol recorded when the acetylant is acetic anhydride and pyridine, that other constituents are acetylized, and ultimately expressed as menthol, by the U. S. P. XI method. An average of 2.4 per cent less total menthol was found when the acetylant was acetic anhydride and pyridine. If in Table II and Table IV the average differences in procedures are compared (Table II, 2.8 per cent; Table IV, 2.4 per cent) it could be concluded that the greatest source of error in the U. S. P. XI procedure is the acetylization of some constituent or constituents other than menthol.

Assay Applied to Pure Menthol.—The present method as given by the Association of Official Agricultural Chemists (12) gives high results. Nelson (4) points out that a probable reason is the difficulty of washing the acetylized menthol free of acetic anhydride and this was also observed by the author. Table V shows the results of the determination carried out on purified menthol.

Table V

Sample	Calculated Per Cent Menthol	A. O. A. C. Method, Per Cent Menthol	Suggested Method, Per Cent Menthol
1	100.0	104.6	100.3
2	100.0	101.7	99.9
3	100.0	101.4	100.1
4	100.0	102.2	100.2
5	100.0	102.3	99.8

The suggested method appears to give more true results than that of the A. O. A. C. determination and requires much less time. Only one gram samples were used, whereas five gram samples are required by the A. O. A. C.

SUMMARY

A method is suggested which is suited for the determination of free menthol in oil of peppermint and in menthol. The time required by the suggested method is about one-half of that for the U. S. P. XI method. The saponification step of the U. S. P. XI method, known to be a source of error, is eliminated, as is the washing of the acetylized oil with dilute sodium carbonate solution. An acetylant mixture of acetic anhydride and pyridine, itself quite stable, is used, esterifying the menthol in thirty minutes. Some evidence is presented which indicates that the U. S. P. XI method of acetylization affects other constituents of oil of peppermint besides menthol.

In the determination of esters in oil of peppermint, the addition of water after saponification was found to produce more accurate results, as only one-third of the amount of indicator is required and the end-point is very sharp and easily observed.

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Some of the Constituents of the Tuber of Coqui (*Cyperus rotundus* L.). III.—The Sugars

*By Conrado F. Asenjo**

The purpose of the present communication is to report on the sugars present in the alcoholic extractive obtained from the tubers of *Cyperus rotundus* L.

EXPERIMENTAL

Extraction and Clarification of the Molasses.—After having subjected the tubers to the process of extraction by continuous percolation with petroleum ether and ether, the marc left was subjected to extraction with 95 per cent alcohol. The alcoholic extractive, a dark brown syrup, amounted to 17.7 per cent of the air-dried tubers. Only part of this syrup was soluble in water; the rest, a resinous mass, remained insoluble. The water-soluble portion reduced Fehling's reagent very readily. After slowly evaporating the water at a temperature of not over 50° C., a dark brown molasses remained which amounted to 50 per cent of the original alcoholic extractive. The molasses thus obtained was clarified by treating with basic lead acetate, neutral lead acetate and, finally, with vegetable charcoal. The excess lead was always precipitated with H_2S. The resulting molasses had a light caramel color.

Proximate Composition of the Molasses.—Reducing sugars were determined by the Drefren-O'Sullivan method (1), and amounted to 51 per cent of the molasses. Reducing sugars, after acid hydrolysis, 55 per cent. Non-reducing sugars by difference, 4 per cent. Aldoses determined by the Willstäter-Schudel titration (2), 41.7 per cent. As the molasses gave a positive Seliwanoff's test, the difference of 9.3 per cent between total reducing sugars and aldoses is probably made up of ketoses. The per cent of water in the molasses was 20 per cent. By difference, then, there was 25 per cent of substances which were neither sugars nor water in the clarified molasses.

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Characterization of the Sugars by the Usual Methods.—The molasses yielded very readily an osazone, m. p. 206–210° C., and a semicarbazone, m. p. 178° C. The second derivative, in particular, pointed to the presence of glucose. A methylphenylosazone, m. p. 167–168° C. was also obtained. Methylphenylfructosazone prepared by us from pure *d*-fructose melted at this same temperature.[1] No depression was observed in the melting point on mixing the two derivatives, therefore the presence of fructose was established.

Characterization of the Aldomonosaccharides in the Molasses as Benzimidazole Derivatives.—Following the procedure of Moore and Link (3), the aldomonosaccharides present in the molasses were oxidized to the corresponding aldonic acids and these, in turn, separated as K and Ba salts. These salts were then condensed with *o*-phenylenediamine to obtain the corresponding benzimidazoles.

Table I.—Constants of Benzimidazole Derivatives

Source	Benzimidazole m. p.	Benzimidazole (α_D^{25})	Hydrochloride, m. p.	Picrate, m. p.
K salt from molasses	215	9.6	180	204
K salt from pure *d*-glucose	215	9.5	180	203
Ba salt from molasses	235	−49.0	230	156
Ba salt from pure *d*-arabinose	235	−49.1	230	158

The presence of *d*-glucose, as the principal sugar in the molasses, was further confirmed as large amounts of K *d*-gluconate were obtained. The isolation of a small amount of Ba salt, which yielded *d*-arabobenzimidazole when condensed with *o*-phenylenediamine, is indicative, as pointed out by Moore and Link (3), of the formation of *d*-arabinose from fructose during the oxidation procedure. Therefore, *d*-arabinose is not present as such in the molasses. Its isolation in the form of a Ba salt only serves further to confirm the presence of fructose in the molasses, a fact already established by the preparation of the methylphenylfructosazone.

For the purpose of finding if small traces of benzimidazole derivates have remained in solution in the filtrates, these were treated with a cupric ammonia solution as recommended by Moore and Link (3). The small amount of benzimidazoles, recovered from the Cu salts obtained from both filtrates, was found to be *d*-glucobenzimidazoles. It was concluded then that *d*-glucose was the only aldomonosaccharide present in the molasses.

[1] The melting point for methylphenylfructosazone reported in Browne's "Sugar Analysis," First ed., p. 622, as well as in other handbooks, is 158–160° C.

SUMMARY

The molasses obtained from the alcoholic extractive of the tubers of *Cyperus rotundus* L. contains the following sugars: 41.7 per cent *d*-glucose, 9.3 per cent *d*-fructose and 4 per cent of non-reducing sugars. No other sugars could be detected.

The author is indebted to Professor Karl P. Link and Dr. Standford Moore of the University of Wisconsin for detailed instructions for carrying out the characterization of the sugars by the benzimidazole procedure, which, at the time this investigation was undertaken, still was unpublished.

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The Patron Saints of Pharmacy*

By Leslie G. Matthews†

From the earliest times man has invoked the help of the supernatural—in prehistoric times no less than in the pagan and Christian eras. Those who in historic times early practiced the art of healing came in direct line from the tribal leaders and priest doctors whose healing powers derived partly from knowledge of their art and partly, if not mainly, from their power over others.

"Faith and works" have been nowhere more typically associated than in the sphere of medicine. Although cures depended upon faith in the personality of the healer, the reputation of the healer would often survive his times. Legends grew into a tradition until the stories became not merely those of simple cures but miraculous feats; associated not only with the healer but with his place of abode, the instruments of his art, his tomb, etc. This was certainly the case with the two chief patrons of pharmacy, St.

* Presented to the Historical Section, A. PH. A., Detroit meeting, 1941.

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Fig. 1.—Portraits of St. Cosmos and St. Damian in the Church of Sta. Maria, Barcelona.

Cosmos and St. Damian; less so for the others who will be mentioned.

The object of this paper is to recall some of the incidents in the lives of those Saints who have been adopted as the patrons of pharmacy. Those most closely associated with pharmacy have been St. Cosmos and St. Damian, St. James, St. Emilien, St. Nicolas and St. Roche. The two most widely recognized are the two brothers, Cosmos and Damian.

While we may know little of their actual lives, the whole of Christendom early recognized their outstanding character and reputation in medicine, pharmacy and surgery. The practice of pharmacy developed early in Arabia and there is a special association therefore in their Arabian origin.

According to the accepted legend, they were born in the third century A. D. They were brought up by Christian parents at Ægea on the Gulf of Issus in Cilicia, Asia Minor, and here they studied medicine.

The portraits of them, in whatever century painted (and the example from the altar piece by Jaime Huguet, first half of XVth Century, in the Church of Sta. Maria, Barcelona, is typical), show a close facial resemblance, Cosmos the elder being usually differentiated in dress from Damian the younger. It may be explained that the physician's dress in the times in which the artists lived, was invariably more elaborate than that of the surgeon as there was considerable difference in the social status of the two. They became famous for their skill in healing, in which the use of drugs largely figured, combined with prayer and faith in the Christian religion. It is this use of drugs which is typified in so many paintings and statues of the Saints—one with a spatula and portable box of salves, the other with a gallipot or drug jar, usually of albarello shape, or with a pestle and mortar. In some portraits the drug jar may be replaced by a flask possibly for urine examination, as in another Spanish painting now in Madrid.

One painting of the late XVIIIth Century is apparently intended to depict St. Cosmos seeking divine guidance on the operation

Fig. 2.—Painting by Huguet Showing the Saints Bleeding to Death in Their Martyrdom.

being performed by St. Damian. The surgical instruments are those of the period of the painting. This sense of direct divine intervention is recalled by an incident in Lord Lister's life (1).

> He wrote to his sister Jane on 3rd March, 1857, after his second operating day: "though I again felt a good deal before the operation, yet I lost all consciousness of the presence of the spectators during its performance, and did it exactly as if no one had been looking on. . . . Just before the operation began, I recollected that there was only one Spectator whom it was important to consider, One present alike in the operating theatre and in the private room; and this consideration gave me increased firmness."

Since 1581 the skulls of the Saints have reposed as relics in the Convent of the St. Claire Nuns in Madrid, where they have been the objects of veneration. In 1935 a scientific examination of them showed that their martyrdom had taken place late in life, *i. e.*, above the age of 50, as shown by the state of obliteration of the sutures.

According to tradition they were martyred by Lysis, Roman Governor of the Province of Cilicia. As Christians they were tried and sentenced to death by drowning, but their bonds were broken by an angel who rescued them from the sea. Although they came out of a fire unscathed and survived shooting by arrows, they were finally beheaded (2). (One of Huguet's pictures of their martyrdom shows them bleeding to death.) To avoid desecration their bodies are said to have been removed for burial to Cyrus in Northern Syria.

It was while the Saints' remains were there that their cult became famous. Miracles were reputed to have taken place and the Emperor Justinian himself sought a cure at their shrine. He is also said to have fortified and beautified the City of Cyrus on this account. It may have been partly due to his cure but perhaps chiefly to gain renown for the new city of Byzantium that Justinian had the relics brought there and a church built in which to enshrine them (3). It is possible to argue that the Saints might never have been heard of had not Justinian's overweening pride in his city as a setting for

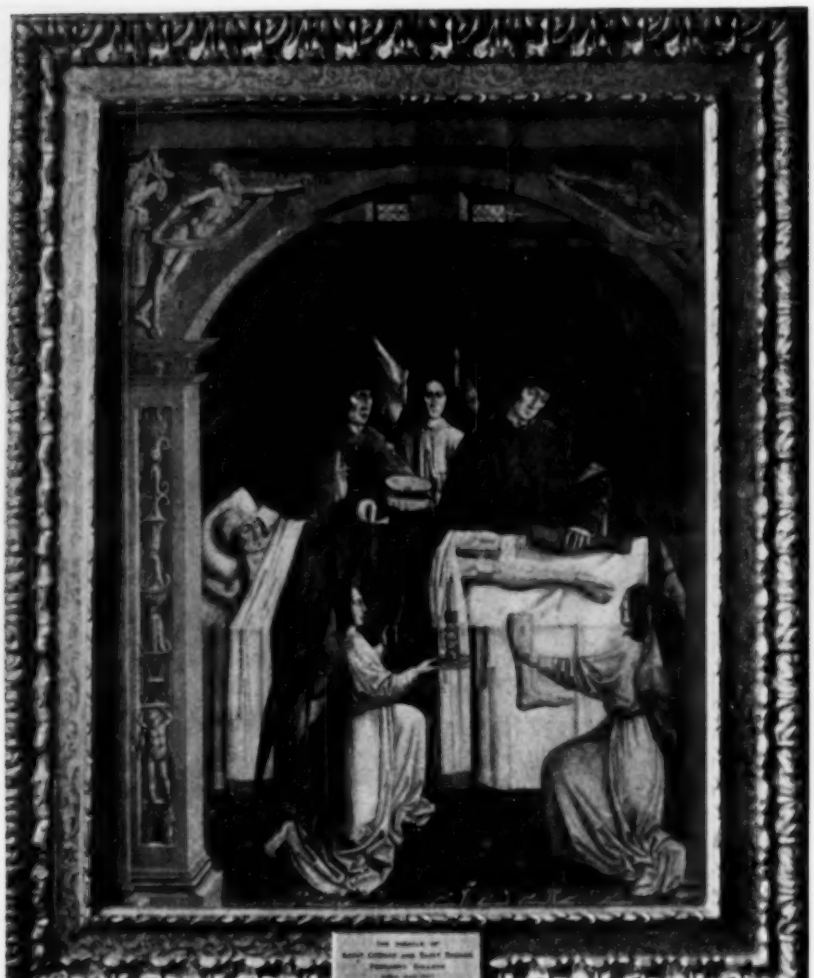

Fig. 3.—Painting by Fernando Gallego, XVth Century Depicting the Saints Operating on a Patient for a Cancerous Condition of a Leg.

himself made him gather there everything that tended to its glory.

The Saints' relics are next heard of in Rome, where in 526–530 A. D. Pope Felix IV had erected a basilica in the Foro Romano, on the site of a former pagan temple dedicated to Romulus. The Pope dedicated the basilica to St. Cosmos and St. Damian, after erecting a tomb in which to deposit their remains which had been transferred from Byzantium. This basilica still stands—it is now the crypt of a church built over it in the XVIIth Century, when the ground level had been raised by about 20 feet.

The tomb may still be seen and on the walls of the apse are some of the most brilliant mosaics in Rome: these were executed in the VIth Century and depict St. Peter and St. Paul presenting St. Cosmos and St. Damian to Christ. For centuries the guild of barbers in Rome has had an annual dedicatory service in the church.

It was in this basilica that there occurred the most famous of the posthumous miracles associated with the saints—the painting by a Spanish artist, Fernando Gallego, XVth Century (now in the Wellcome Historical Medical Museum, London) tells the story. It shows the two Saints operating on a patient who had sought relief from a cancerous condition of the leg. The story goes that having travelled far he came late to the church and found the doors locked against him—after praying to the saints he was over-

come with sleep and dreamed that they appeared to him, examined his leg and decided the disease was too far advanced for treatment to be effective. The Saints not to discourage the faith of the suppliant sought in the cemetery of the Via Appia the body of a man newly buried, whose sound leg they proposed to graft, after amputating the diseased leg of the patient. To the patient's surprise, when he awakened, he had two sound legs but the replaced leg was black. The Saints apparently had been indifferent to the fact that their grafting had been that of the leg of a Moor.

H. Peters (4) suggests it is the effectual grafting of the Moor's leg for this miracle that gave rise in the middle ages to the "Sign of the Moor" as a favorite exterior apothecaries' sign—a black Moor. "Apoteken zum Mohren" is a frequent name for Mid-European pharmacies.

The painters of this subject had full scope for the realistic treatment of martyrdom. Gallego's painting shows St. Cosmos about to graft the black leg—the patient meanwhile peaceful and apparently under the influence of a narcotic.

The reference to the patient's sleep at the church is an interesting example of the carrying over into the early centuries of the Christian era of the principles of pagan "temple incubation sleep." This is recorded as a frequent form of treatment at the temples of Asklepios in Greece: the patient slept in the temple, the god appeared to him in a vision and healing resulted.

In Huguet's version of the subject (Spanish, first half of XVth Century, now in Barcelona), both Cosmos and Damian are engaged in grafting the Moor's leg. The two attendant angels hold the gallipot and box of salves associated with the Saints; others in the background attend to the Moor. This picture may have been intended to portray the surgeon craft of the Saints.

In another by Huguet, the donor of which may have been a Master Apothecary, the Saints themselves are shown with gallipot and salves.

There are many other versions of the miracle, many of them Spanish but two panels of a tryptich in the Antwerp Museum (Ambrosius Francken, late XVIth Century) show the martyrdom and the miracle.

The cult of the Saints extended throughout Christendom. In England the records though scanty are widespread. Three churches were dedicated to the Saints; there were statues erected to their honor on the West Front of Salisbury Cathedral (XIIIth Century) and a XVth Century painted reed screen (Wolborough Church, Devon) pictures the Saints—Cosmos with unguent jar and spatula, and Damian with a flask. Relics were held in the Treasury of Christ Church, Canterbury. Official medicine recognized them: they were adopted as the Patron Saints of the Barber-Surgeons, and they figured as Supporters on the Coat of Arms of the Company of Surgeons, granted in 1492. The lack of recognition subsequently may have been caused by the break with Rome in Henry VIII's time.

In Italy and Spain the healing powers of the Saints were constantly invoked and their patronage recognized.* In Spain as late as 1918, broadsheets were being printed for distribution in Catalonia on the Saints' Day —27th September. Copies of these broadsheets (from a famous collection, now in the Wellcome Museum) depict the Saints with drug jars, spatula and salves. All versions of the story, as told in the verses printed on the sheets, contain the principal elements of the legends of their life, martyrdom and miracles. These broadsheets besides telling the lives of the Saints also included the invocation and prayers to be used—occasionally also the music of the hymn.

Reference has been made to the relics in Madrid—according to a well-documented account (5) the remains of the Saints were removed by Bishop Adaldago from the Basilica in Rome to Bremen (in the Xth Century); later Emperor Henry of the Holy Roman Empire had the skulls taken to Bamburg in Germany. Finally, in 1581, they were removed to Madrid by order of the Empress Maria, daughter of Charles V. Photographs show the skulls, wrapped at

* In Italy the Saints were the patrons of the Medici family and as such appeared on the coins of Florence.

Fig. 4.—Wood Carvings of the Saints in the Pharmaceutical Museum of Basle, Switzerland.

the base in red silk and kept in position with silver bands bearing Greek inscriptions by which they were identified.

In France Cosmos and Damian have been long adopted by pharmacists as patrons of their craft.

In Switzerland busts or figures in wood, sometimes gilded, were found in many pharmacies. Some fine examples are in the Basle pharmaceutical Museum (6), an indication of the recognition there of the Saints' patronage.

In Austria the Saints were recognized as patrons of medicine and pharmacy. The Vienna Medical Society possessed a copy of a card for 1700, inviting physicians, students, druggists and surgeons to take part in a festival celebration on the Saints' Day in St. Stephen's Cathedral, and also had copies of the plates from which the pictures were printed for distribution to those participating (7).

It is recorded that in Greece more than fifty churches were dedicated to the Saints, whose reputation in that country was particularly high.

The records available in Europe show clearly the association of SS. Cosmos and Damian with pharmacy to be a strong one, and one that has been maintained, in catholic countries at least, throughout the centuries.

Of the saints whose patronage is not so closely restricted to pharmacy but who have a definite association with apothecaries, pharmacists and druggists, the following should be mentioned: St. James, St. Nicolas, St. Roche, and St. Emilien.

Saint James, the brother of John, son of Zebedee; the patron saint of apothecaries and druggists, but also of pilgrims, polishers and shoemakers. Perhaps chiefly regarded as the patron of pilgrims since he is so usually depicted with pilgrim staff and scollop shells. The Church of St. James of Compostella in Spain (of which country he is the Patron Saint) is decorated with scollop shells which since that time have been adopted as a pilgrim badge. He is commemorated on the 26th July.

Saint Nicolas, IVth Century. Archbishop of Mire (Saint's Day 6th December). Perhaps no Saint is more highly regarded or invoked by children the world over. Notwithstanding their special claims on him—as shown by his release of three of them from the salt-tub (usually to be seen in

illustrations)—almost the world is his parish, for he is also patron of scholars, school-masters, sailors, butchers, as well as of perfumers and pharmacists. It is of interest that he was adopted as Patron by the Master Apothecaries of Paris.

Saint Roche, born in Montpellier in 1295. His ability to heal the plague from which he himself was cured, when neither physician nor apothecary knew even what to prescribe, naturally caused them to return to him as their Patron. He is shown with a plague spot on his knee, whose cure was assisted by an angel, and whose food, during his banishment on account of the plague, was brought by a dog—hence the picture always of a dog bringing a cake of bread in his mouth. Celebrated on the 16th August.

Saint Emilien, Vth Century, was a physician who suffered tortures during the persecution of the Vandals, under King Huneric. Saint's Day 6th December.

Other Saints were chosen according to special local circumstances, sometimes connected with the Church where the corporation or confraternity of apothecaries or pharmacists held its annual service, and whose name was inscribed on the banner of the Guild—one of the banners illustrated in Bouvet's "Histoire de la pharmacie en France" is that of the Lille Apothecaries whose choice was Saint Madeleine.

Had the Centenary of the Pharmaceutical Society of Great Britain (April 1941) occurred in an earlier century, no doubt at the commemorative service the banners of the Society, emblazoned with the pictures of St. Cosmos and St. Damian, at least, would have been carried in procession round the church in recognition of their patronage of the profession of pharmacy.

I wish to acknowledge the facilities afforded by the Director of the Wellcome Historical Medical Museum (Dr. S. H. Daukes) and also the assistance given in the preparation of this paper by Mr. P. Johnston-Saint, the Conservator who, having visited some of the shrines, has been able to give first-hand information about them.

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Book Reviews

Semimicro Qualitative Analysis, by ARTHUR R. MIDDLETON, Ph.D., Professor of Inorganic Chemistry, Purdue University, and JOHN W. WILLARD, B.S. xi + 446 pp., 7 figs., 23 tables, 14.6 x 22.7 cm. 1939. Prentice-Hall, Inc., 70 Fifth Avenue, New York, New York. Price, $3.50.

This is a laboratory manual combining theory and laboratory work under a single cover. The methods included in the text are the result of a six-year trial in large student classes. According to the authors, the methods described have given exceptionally good results. The advantages of using semimicro quantities for qualitative analyses are stressed.

This book features two ideas of qualitative analysis which have hitherto not appeared in any text. Anions are determined qualitatively by elimination; and analysis of cation group III is performed without removal of arsenate and phosphate, and of cation group IV without the use of the rather unsatisfactory reagent, ammonium carbonate. Much time is saved in the laboratory by the introduction of these new methods.

The book is divided into two parts: Part I deals with the theory of chemistry and Part II presents the laboratory methods.

The section on theory explains the use of units in chemical calculation and the application of logarithms to simplify calculations. Other subjects treated in succession are application of the gas laws, structure of matter, laws of equilibrium, homogeneous equilibrium in aqueous solution, heterogeneous equilibrium, hydrolysis, equilibrium of complex ions, and equilibrium in redox reactions (reduction, oxidation and potential).

The section on laboratory work is divided into twelve chapters: namely, semimicro technique, properties and reactions of the cations, preparation for the laboratory work, introductory study of the cations, systematic analysis of cations, introduction to the analysis of anions, properties and reactions of

the anions, introductory study of anions, systematic analysis of anions, systematic qualitative analysis, and analysis of non-ferrous alloys.—EMERSON C. BEELER.

Temperature, Its Measurement and Control in Science and Industry. xii + 1362 pp., 550 figs. 1941. New York: Reinhold Publishing Corporation. Price, $11.00.

This book is a collection of 126 papers on temperature measurement and control, originally presented at a symposium held under the auspices of the American Institute of Physics and coöperating scientific and technical societies, in November 1939. The papers cover problems in the application of temperature measurement and control in a wide variety of fields, including physics, chemistry, engineering, metallurgy, medicine, biology and their various specialized branches. The work is, in effect, a comprehensive treatise on temperature, including both the practical and theoretical sides of the subject. An appendix of 30 pages of useful thermometric data is included, and also a well-prepared subject index.

The papers are arranged in chapters dealing with the following topics: basis of temperature scales; precision thermometry, including resistance, thermoelectric and liquid in glass; teaching of thermometry; temperature in geology and meteorology; temperature in biology; temperature and its regulation in man; devices and methods for automatic temperature control; special miscellaneous applications and methods, very low and very high temperatures; general engineering, including applications in air conditioning, refrigeration, steam engineering and aircraft; metallurgy and ceramics; oil industries; optical and radiation pyrometry; thermometric metals and alloys, mainly calibration and life data.—M. S. VAN DUSEN.

Books Received

To January 1, 1942

(Continued from January Issue)

HERMAN C. NOLEN and HAROLD H. MAYNARD, *Drug Store Management.* McGraw-Hill Book Company, Inc., New York, N. Y., 1941. 570 pp. $4.00.

HERMAN PRINZ and U. GARFIELD RICKERT, *Pharmacology and Dental Therapeutics.* Eighth edition, revised by EDWARD C. DOBBS. The C. V. Mosby Company, St. Louis, Mo., 1941. 507 pp. $6.50.

HENRY E. SIGERIST, *Medicine and Human Welfare.* Yale University Press, New Haven, Conn., 1941. 148 pp. $2.50.

CLAYTON S. SMITH and HELEN L. WIKOFF, *Materia Medica and Pharmacology,* an Introductory Text. College Book Company, Columbus, Ohio, 1941. 334 pp.

T. B. SMITH, *Analytical Processes, a Physico-Chemical Interpretation.* Second edition. Longmans, Green and Company, Inc., 55 Fifth Avenue, New York, N. Y., 1940. 470 pp. $5.00.

WESLEY W. SPINK, *Sulfanilamide and Related Compounds in General Practice.* The Year Book Publishers, Inc., 304 South Dearborn Street, Chicago, Ill., 1941. 256 pp.

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A. G. WOODMAN, *Food Analysis, Typical Methods and the Interpretation of Results.* Fourth edition. McGraw-Hill Book Company, Inc., New York, N. Y., 1941. 607 pp. $4.00.